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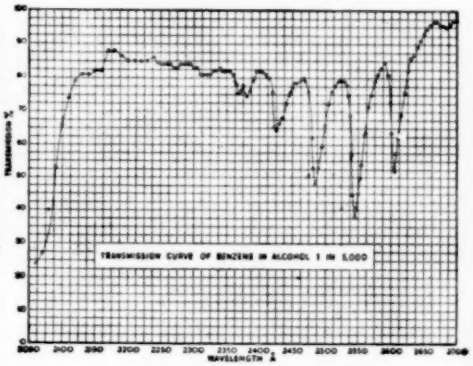

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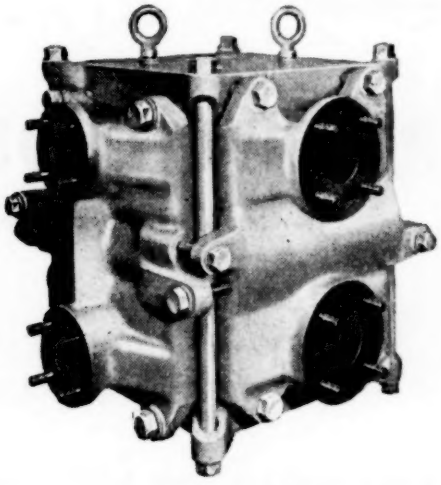

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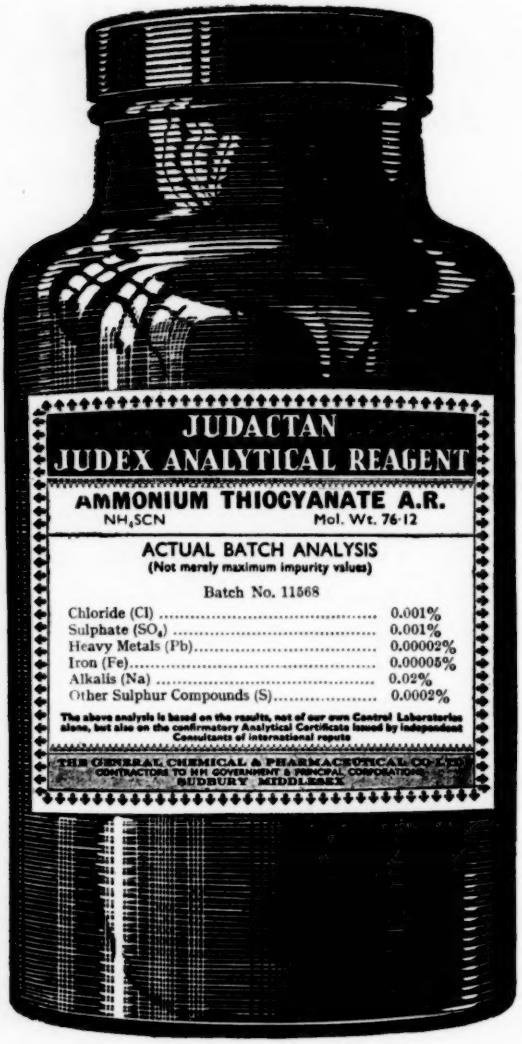
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JOURNAL
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1. *New Syntheses of Heterocyclic Compounds. Part XIII.* Some* 10-*Amino*-1 : 3-*dimethyl*-2 : 9-*diazaphenanthrenes.*†

By A. Courts and V. Petrow.

Methods for the preparation of 2 : 9-diazaphenanthrenes, developed in Part VII of this series, have been extended to include the corresponding 10-amino-4-cyano- and 4-acetyl-10-methyl derivatives.

Biological study of the diazaphenanthrenes described in Parts V and VII of this series (Petrow, *J.*, 1946, 200, 884) has revealed that certain of these compounds show marked analeptic properties (Thorp, Thesis, London, 1947). It was, therefore, of interest to extend the chemistry of this group of compounds in order to throw further light on the structural features associated with this type of biological action. With this object in view, we have applied the synthetic methods developed in Part VII to the preparation of some new 10-amino-4-cyano-2 : 9-diazaphenanthrenes † (IV; R′ = CN, R = NH_2). In addition, we have elaborated a route whereby derivatives of the hitherto unknown 10-methyl-2 : 9-diazaphenanthrene may be obtained.

The preparation of 4-aryl-3 : 5-dicyano-2 : 6-dimethyldihydropyridines (cf. II) had been described by Meyer (*J. pr. Chem.*, 1895, **52**, 101; 1908, **78**, 507) and Mohr (*ibid.*, 1897, **56**, 124), who obtained such compounds by condensing β-aminocrotonitrile with an aryl aldehyde in acidified ethanolic solution (cf. I). When *o*-nitrobenzaldehyde was employed for this purpose only moderate yields (*ca.* 20%) of the resulting 3 : 5-dicyano-1 : 4-dihydro-2 : 6-dimethyl-4-*o*-nitrophenylpyridine (II) were obtained. Systematic study of the reaction conditions, however, revealed that the yield of required material could be raised to 88% by carrying out the condensation in glacial acetic acid solution, and this improved procedure was employed throughout the investigation. In this way, the corresponding 4-*o*-nitrophenyl-2 : 6-diphenyl, 4-(2 : 4-dinitrophenyl)-2 : 6-dimethyl, 4-(2 : 4-dinitrophenyl)-2 : 6-diphenyl, 4-(4-hydroxy-3-methoxy-2-nitrophenyl)-2 : 6-dimethyl, and 4-(4-hydroxy-3-methoxy-2-nitrophenyl)-2 : 6-diphenyl analogues of (II) were readily obtained.

Oxidation of the foregoing 1 : 4-dihydropyridines to the corresponding pyridines (cf. III) was effected by means of dilute nitric acid, or, better, by chromic acid in hot acetic acid solution or suspension (Meyer, *J. pr. Chem.*, 1915, **92**, 175). Phenolic groups were protected by acetylation before oxidation. Yields were uniformly good.

Reductive cyclisation of (III) with reduced iron in acidulated aqueous-ethanolic solution surprisingly failed to give 10-amino-4-cyano-1 : 3-dimethyl-2 : 9-diazaphenanthrene (cf. IV; R = NH_2, R′ = CN), a result in marked contrast to observations recorded in Part VII. The latter compound was ultimately obtained by performing the reduction with hot sodium dithionite (hydrosulphite) solution. Reductive cyclisation of the remaining *o*-nitrophenylpyridines likewise required individual study of each compound, the 10-amino-4-cyano-2 : 9-diazaphenanthrenes being obtained by use of the reducing agents indicated : 1 : 3-diphenyl- (stannous chloride), 7-amino-1 : 3-dimethyl- (reduced iron), 7-acetoxy-8-methoxy-1 : 3-dimethyl- (reduced iron), 7-acetoxy-8-methoxy-1 : 3-diphenyl- (reduced

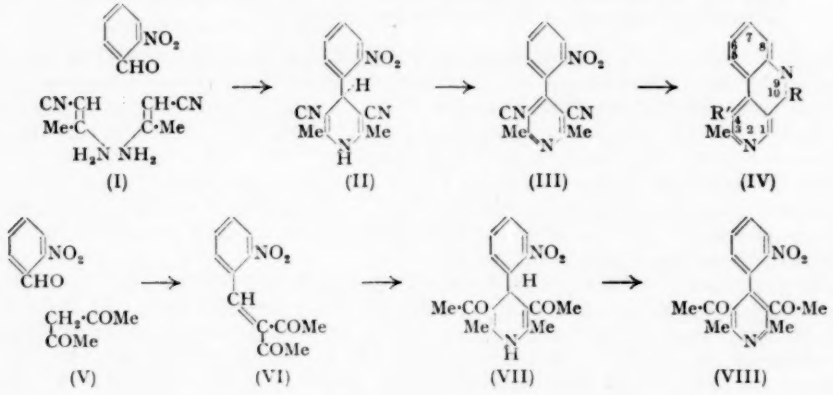
* Part XII, *J.*, 1951, 551.

† Referred to as 7 : 10-diazaphenanthrenes in Part VII for reasons outlined in Part V.

iron). Experiments on the reductive cyclisation of 3 : 5-dicyano-4-(2 : 4-dinitrophenyl)-2 : 6-diphenylpyridine proved inconclusive.

The preparation of a 9-methyl-2 : 10-diazaphenanthrene has been described in Part V (*loc. cit.*). Some isomeric 10-methyl-2 : 9-diazaphenanthrenes (IV; R = Me) have now been prepared by reductive cyclisation of suitably constituted 3 : 5-diacetyl-4-*o*-nitrophenylpyridines (VIII), which were obtained by means of a Knoevenagel extension of the Hantzsch synthesis.

Ruhemann (*J.*, 1903, **83**, 1373) has shown that *m*-nitrobenzaldehyde reacts with acetylacetone, in the presence of piperidine as catalyst, to give *m*-nitrobenzylideneacetylacetone. By use of *o*-nitrobenzaldehyde, *o*-nitrobenzylideneacetylacetone (VI) has been obtained in nearly quantitative yield. The latter compound (VI) has now been found to undergo facile condensation with 4-aminopent-3-en-2-one (the " ammonia derivative " of acetylacetone) at 100° to give 3 : 5-diacetyl-1 : 4-dihydro-2 : 6-dimethyl-4-*o*-nitrophenylpyridine (VII). Oxidation of the dihydropyridine with chromium trioxide furnished the corresponding pyridine derivative (VIII), which passed smoothly into 4-acetyl-1 : 3 : 10-trimethyl-2 : 9-diazaphenanthrene on reduction with reduced iron.



Attempts to prepare (VII) by direct condensation of *o*-nitrobenzaldehyde with acetylacetone and its " ammonia " derivative proved unsuccessful, preliminary formation of (VI) being required. With 2-nitrovanillin as the carbonyl component, however, a different situation obtained. In this instance attempts to prepare the initial condensation product corresponding to (VI) were not successful. 3 : 5-Diacetyl-1 : 4-dihydro-4-(4-hydroxy-3-methoxy-2-nitrophenyl)-2 : 6-dimethylpyridine was ultimately prepared in low yield by simply heating the three components in ethanolic solution on the water-bath. Oxidation of its acetyl derivative, followed by reduction, furnished 7-acetoxy-4-acetyl-8-methoxy-1 : 3 : 10-trimethyl-2 : 9-diazaphenanthrene, hydrolysis of which gave the 7-hydroxy-derivative which could be methylated in the usual way.

In addition to studying the reductive ring closure of compounds of types (III) and (VIII), an attempt has been made to extend the work to include some 4-*o*-nitrophenylpyridines possessing two different groups at 3 and 5. Some preliminary experiments in this direction have already been reported in Part VII, in which the conversion of 5-carbethoxy-3-cyano-2 : 6-dimethyl-4-*o*-nitrophenylpyridine into 10-amino-4-carbethoxy-1 : 3-dimethyl-2 : 9-diazaphenanthrene has been described.

Condensation of *o*-nitrobenzylideneacetylacetone with ethyl β-aminocrotonate furnished 5-acetyl-3-carbethoxy-1 : 4-dihydro-2 : 6-dimethyl-4-*o*-nitrophenylpyridine in 42% yield. Oxidation of this compound gave a gummy product which could not be crystallised. It was, therefore, reduced directly with reduced iron to give 5-acetyl-4-*o*-aminophenyl-3-carbethoxy-2 : 6-dimethylpyridine, a relatively stable compound which did not appear to undergo spontaneous ring closure. Treatment with acetic anhydride effected lactam

formation with concomitant acetylation, to give 10-acetoxy-4-acetyl-1 : 3-dimethyl-2 : 9-diazaphenanthrene, which was isolated as the hemihydrate.

Condensation of *o*-nitrobenzylideneacetylacetone with β-aminocrotononitrile gave 5-acetyl 3-cyano-1 : 4-dihydro-2 : 6-dimethyl-4-*o*-nitrophenylpyridine, which could not be obtained analytically pure. Oxidation furnished the pyridine derivative, reduction of which gave an inseparable mixture of products in which the corresponding diazaphenanthrenes appeared to be present.

Experimental

(M. p.s are uncorrected. Microanalyses are by Drs. Weiler and Strauss.)

3 : 5-*Dicyano*-1 : 4-*dihydro*-2 : 6-*dimethyl*-4-*o*-*nitrophenylpyridine* (II).—A solution of *o*-nitrobenzaldehyde (4·5 g.) and β-aminocrotononitrile (4·9 g.) (Holtzwart, *J. pr. Chem.*, 1889, **39**, 230) in glacial acetic acid (25 ml.) was heated under reflux for 15 minutes. Separation of solid began almost immediately. After cooling, the solids were collected and crystallised from absolute ethanol, to give the *dicyano*-compound (II), canary-yellow needles, m. p. 216—217° (Found : C, 63·5; H, 4·8; N, 19·9. $C_{15}H_{12}O_2N_4$ requires C, 64·3; H, 4·3; N, 20·0%).

3 : 5-*Dicyano*-2 : 6-*dimethyl*-4-*o*-*nitrophenylpyridine* (III).—The foregoing compound (4 g.) in hot acetic acid (20 ml.) was oxidised with chromic acid (1 g.) dissolved in a little water and added dropwise with stirring. **3 : 5-*Dicyano*-2 : 6-*dimethyl*-4-*o*-*nitrophenylpyridine*** separated on cooling in nearly quantitative yield and, after crystallisation from aqueous ethanol, formed faintly yellow needles, m. p. 146·5—147·5° (Found : C, 64·8; H, 3·4; N, 19·7. $C_{15}H_{10}O_2N_4$ requires C, 64·8; H, 3·6; N, 20·1%).

10-*Amino*-4-*cyano*-1 : 3-*dimethyl*-2 : 9-*diazaphenanthrene*.—The foregoing compound (2 g.) was finely powdered and added in one portion to a hot concentrated solution (25 ml.) of sodium dithionite (hydrosulphite). After 2 hours on the water-bath the cooled mixture was extracted with chloroform, and the extract evaporated to dryness. Crystallisation from absolute ethanol gave **10-*amino*-4-*cyano*-1 : 3-*dimethyl*-2 : 9-*diazaphenanthrene*** (47%), light yellow needles, m. p. 273° (Found : C, 72·5; H, 5·1; N, 22·4. $C_{15}H_{12}N_4$ requires C, 72·6; H, 4·8; N, 22·6%). The compound failed to give the primary amine test on diazotisation and coupling with alkaline β-naphthol, a behaviour characteristic of this type of compound (cf. Parts V and VII, *locc. cit.*).

3 : 5-*Dicyano*-1 : 4-*dihydro*-4-*o*-*nitrophenyl*-2 : 6-*diphenylpyridine*.—This was prepared by condensing *o*-nitrobenzaldehyde (3 g.) and β-aminocinnamonitrile (6 g.) (Holtzwart, *loc. cit.*) in glacial acetic acid (25 ml.) under reflux, in nearly quantitative yield and had m. p. 258° (cf. Meyer, *J. pr. Chem.*, 1908, **78**, 512).

3 : 5-*Dicyano*-4-*o*-*nitrophenyl*-2 : 6-*diphenylpyridine*.—The foregoing dihydro-compound (5·9 g.) was finely powdered and suspended in hot acetic acid (100 ml.). To it, chromium trioxide (1·2 g.) dissolved in water (*ca.* 5 ml.) was rapidly added in one portion. The dihydro-base went rapidly into solution, and **3 : 5-*dicyano*-4-*o*-*nitrophenyl*-2 : 6-*diphenylpyridine*** immediately separated in nearly quantitative yield, as felted white needles (from glacial acetic acid), m. p. 232—233° (Found : C, 74·7; H, 3·6; N, 13·8. $C_{25}H_{14}O_2N_4$ requires C, 74·6; H, 3·5; N, 13·9%).

10-*Amino*-4-*cyano*-1 : 3-*diphenyl*-2 : 9-*diazaphenanthrene*.—The foregoing compound (2 g.) was suspended in a mixture of concentrated hydrochloric acid (80 ml.) and glacial acetic acid (40 ml.) and treated with stannous chloride dihydrate (16 g.). The mixture was then heated on the water-bath until all the original base had disappeared and had been replaced by a yellow crystalline deposit. After being kept overnight, the product was collected, digested with excess of concentrated sodium hydroxide solution, and allowed to cool. The precipitated solids, on crystallisation from pyridine, yielded **10-*amino*-4-*cyano*-1 : 3-*diphenyl*-2 : 9-*diazaphenanthrene***, felted, golden-yellow needles, m. p. 276—277° (Found : C, 80·2; H, 4·4; N, 15·3. $C_{25}H_{16}N_4$ requires C, 80·6; H, 4·3; N, 15·1%). The compound formed a sparingly soluble hydrochloride which failed to give a primary amine test on diazotisation.

3 : 5-*Dicyano*-1 : 4-*dihydro*-4-(2 : 4-*dinitrophenyl*)-2 : 6-*dimethylpyridine*, obtained in nearly quantitative yield, formed lemon-yellow needles, m. p. 268—270° (decomp.), on crystallisation from a very large volume (300 pts.) of glacial acetic acid (Found : C, 55·6; H, 3·5; N, 21·2. $C_{15}H_{11}O_4N_5$ requires C, 55·4; H, 3·4; N, 21·5%).

3 : 5-*Dicyano*-4-(2 : 4-*dinitrophenyl*)-2 : 6-*dimethylpyridine* (78%) separated from absolute ethanol in faintly yellow pyramids, m. p. 159·5° (Found : C, 55·3; H, 2·9; N, 21·4. $C_{15}H_9O_4N_5$ requires C, 55·5; H, 2·8; N, 21·7%).

7 : 10-*Diamino*-4-*cyano*-1 : 3-*dimethyl*-2 : 9-*diazaphenanthrene*.—The foregoing compound

(1 g.) was heated under reflux for 1 hour with ethanol (30 ml.) containing 3 drops of concentrated hydrochloric acid and reduced iron (3 g.). After removal of solids a few drops of ammonia solution (*d* 0·88) were added, and the mixture was boiled and filtered. Concentration to small bulk gave **7 : 10-*diamino*-4-*cyano*-1 : 3-*dimethyl*-2 : 9-*diazaphenanthrene hemihydrate***, deep yellow crystals, m. p. 288—289° (decomp.) (Found : C, 66·1; H, 5·1; N, 25·0. $C_{15}H_{13}N_5,\frac{1}{2}H_2O$ requires C, 66·2; H, 5·1; N, 25·1%).

3 : 5-*Dicyano*-4-(2 : 4-*dinitrophenyl*)-1 : 4-*dihydro*-2 : 6-*diphenylpyridine*.—2 : 4-Dinitrobenzaldehyde (4 g.), β-aminocinnamonitrile (6 g.), and glacial acetic acid (25 ml.) were heated under reflux for 1 hour. Addition of water precipitated an orange gum which solidified in contact with absolute ethanol (10 ml.). Crystallisation from absolute ethanol or glacial acetic acid gave **3 : 5-*dicyano*-4-(2 : 4-*dinitrophenyl*)-1 : 4-*dihydro*-2 : 6-*diphenylpyridine*** (45%), small yellow plates, m. p. 218° (Found : C, 66·8; H, 3·2; N, 15·7. $C_{25}H_{15}O_4N_5$ requires C, 66·8; H, 3·3; N, 15·6%). **3 : 5-*Dicyano*-4-(2 : 4-*dinitrophenyl*)-2 : 6-*diphenylpyridine***, obtained in nearly quantitative yield, formed colourless needles (from absolute ethanol), m. p. 211° (Found : C, 66·7; H, 2·8; N, 15·6. $C_{25}H_{13}O_4N_5$ requires C, 67·1; H, 2·9; N, 15·6%). Catalytic reduction of this compound (1 g.) in absolute ethanol (300 ml.) in the presence of palladised charcoal (0·75 g.) gave small orange needles, m. p. >300° (Found, in 4 samples : C, 68·0—70·4; H, 4·1—4·4; N, 14·1—15·2%).

3 : 5-*Dicyano*-1 : 4-*dihydro*-4-(4-*hydroxy*-3-*methoxy*-2-*nitrophenyl*)-2 : 6-*dimethylpyridine*, obtained (70%) from 2-nitrovanillin (Stoessen, *J. Amer. Chem. Soc.*, 1928, **50**, 2559), separated from glacial acetic acid in faintly yellow needles, m. p. 266° (decomp.) (Found : C, 58·9; H, 4·3; N, 17·2. $C_{16}H_{14}O_4N_4$ requires C, 58·9; H, 4·3; N, 17·2%). **4-(4-*Acetoxy*-3-*methoxy*-2-*nitrophenyl*)-3 : 5-*dicyano*-1 : 4-*dihydro*-2 : 6-*dimethylpyridine***, obtained (80%) by acetylation of the foregoing compound (2 g.) with acetic anhydride (15 ml.) for 30 minutes under reflux, formed pale yellow needles, m. p. 214°, from glacial acetic acid (Found : C, 58·8; H, 4·5. $C_{18}H_{16}O_5N_4$ requires C, 58·7; H, 4·3%). **4-(4-*Acetoxy*-3-*methoxy*-4-*nitrophenyl*)-3 : 5-*dicyano*-2 : 6-*dimethylpyridine***, obtained (80%) by oxidation of the foregoing compound, formed large glistening plates (from absolute ethanol), m. p. 190·5° (Found : C, 59·0; H, 4·2; N, 15·3. $C_{18}H_{14}O_5N_4$ requires C, 59·0; H, 3·8; N, 15·3%). **7-*Acetoxy*-10-*amino*-4-*cyano*-8-*methoxy*-1 : 3-*dimethyl*-2 : 9-*diazaphenanthrene***, obtained (80%) by reducing the foregoing compound with reduced iron, formed lemon-yellow needles (from ethanol), m. p. 239° (Found : C, 64·7; H, 4·7; N, 16·4. $C_{18}H_{18}O_3N_4$ requires C, 64·3; H, 4·8; N, 16·7%).

3 : 5-*Dicyano*-1 : 4-*dihydro*-4-(4-*hydroxy*-3-*methoxy*-2-*nitrophenyl*)-2 : 6-*diphenylpyridine* separated (70%) from glacial acetic acid in very small phototropic crystals, m. p. 279° (Found : C, 67·9; H, 4·1; N, 12·2. $C_{26}H_{18}O_4N_4,\frac{1}{2}H_2O$ requires C, 68·0; H, 4·1; N, 12·2%). Its *acetyl* derivative (82%) formed long, pale-yellow needles (from absolute ethanol), m. p. 242° (Found : C, 67·9; H, 4·4. $C_{28}H_{20}O_5N_4$ requires C, 68·3; H, 4·1%).

4-(4-*Acetoxy*-3-*methoxy*-2-*nitrophenyl*)-3 : 5-*dicyanopyridine* (70%) separated from alcohol in small needles, m. p. 184—185° (Found : C, 69·0; H, 3·6; N, 11·4. $C_{23}H_{18}O_5N_4$ requires C, 68·6; H, 3·7; N, 11·4%).

7-*Acetoxy*-10-*amino*-4-*cyano*-1-*methoxy*-1 : 3-*diphenyl*-2 : 9-*diazaphenanthrene* (>90%) crystallised from ethanol in pale-yellow needles, m. p. 253° (Found : C, 73·0; H, 4·4; N, 12·3. $C_{28}H_{20}O_3N_4$ requires C, 73·0; H, 4·3; N, 12·1%).

o-*Nitrobenzylideneacetylacetone* (VI), obtained by leaving a solution of powdered *o*-nitrobenzaldehyde (7·5 g.) in acetylacetone (5 g.) containing a little piperidine for 2 days in a desiccator, formed pale yellow plates, m. p. 75·5—76·5°, from ethanol (Found : C, 61·8; H, 4·9; N, 5·7. $C_{12}H_{11}O_4N$ requires C, 61·8; H, 4·7; N, 6·0%).

3 : 5-*Diacetyl*-1 : 4-*dihydro*-2 : 6-*dimethyl*-4-o-*nitrophenylpyridine* (VII).—The foregoing compound (7·5 g.) and 4-aminopent-3-en-2-one (6·5 g.) (Combes and Combes, *Bull. Soc. chim.*, 1892, **7**, 779) were heated together on the water-bath for 12 hours. The partly crystalline product obtained was freed from oily material by addition of methanol (10 ml.), the solids being collected after 12 hours and crystallised from a little ethanol. The *diacetyl* compound (VII) (32%) formed phototropic yellow needles, m. p. 226·5—227·5° (Found : C, 64·6; H, 5·6; N, 8·8. $C_{17}H_{18}O_4N_2$ requires C, 65·0; H, 5·1; N, 8·9%). Addition of a few drops of piperidine to the reaction mixture lowered the yield to 27%. The corresponding *pyridine* (VIII) (>95%) formed nearly colourless needles (from aqueous alcohol), m. p. 142·5—143·5° (Found : N, 9·3. $C_{17}H_{16}O_4N_2$ requires N, 9·0%).

4-*Acetyl*-1 : 3 : 10-*trimethyl*-2 : 9-*diazaphenanthrene* (IV; R = Me, R′ = Ac) (45—50%), spear-head-shaped crystals from ligroin, had m. p. 166—167° (Found : C, 77·5; H, 5·7; N, 10·4. $C_{17}H_{16}ON_2$ requires C, 77·3; H, 6·0; N, 10·6%).

3 : 5-*Diacetyl*-1 : 4-*dihydro*-4-(4-*hydroxy*-3-*methoxy*-2-*nitrophenyl*)-2 : 6-*dimethylpyridine*, prepared (21%) by heating 2-nitrovanillin (19 g.), acetylacetone (12·5 ml.), and 4-aminopent-3-en-2-one (12·5 ml. in (ethanol (60 ml.) in an open flask on the water-bath for 2 days, followed by stirring with cold methanol (60 ml.), formed ivory needles from ethanol; it had m. p. 246—247° (decomp.) (Found: C, 59·8; H, 5·5; N, 7·5. $C_{18}H_{20}O_6N_2$ requires C, 60·0; H, 5·6; N, 7·8%). It failed to give a colour with ferric chloride. Its *acetyl* derivative formed very small needles (from acetic acid), m. p. 254·5—255·5° (decomp.) (Found: C, 59·3; H, 5·3; N, 6·3. $C_{20}H_{22}O_7N_2$ requires C, 59·7; H, 5·5; N, 7·0%).

4-(4-*Acetoxy*-3-*methoxy*-2-*nitrophenyl*)-3 : 5-*diacetyl*-2 6-*dimethylpyridine* (69%), plates from aqueous acetone, had m. p. 152·5—153·5° (Found: C, 60·0; H, 4·7; N, 7·3. $C_{20}H_{20}O_7N_2$ requires C, 60·0; H, 5·0; N, 7·0%).

7-*Acetoxy*-4-*acetyl*-8-*methoxy*-1 : 3 : 10-*trimethyl*-2 : 9-*diazaphenanthrene* (82%) was obtained in faintly yellow plates, m. p. 153·5—154·5° (Found: C, 68·4; H, 5·6; N, 7·5. $C_{20}H_{20}O_4N_2$ requires C, 68·2; H, 5·7; N, 8·0%). **4-*Acetyl*-7-*hydroxy*-8-*methoxy*-1 : 3 : 10-*trimethyl*-2 : 9-*diazaphenanthrene***, prepared by hydrolysis of the foregoing compound with 10% potassium hydroxide solution (10 vols.) followed by precipitation with carbon dioxide, formed octahedra from ethanol–ligroin and had m. p. 202—203° (Found: C, 70·0; H, 5·5; N, 9·0. $C_{18}H_{18}O_3N_2$ requires C, 69·7; H, 5·8; N, 9·0%). **4-*Acetyl*-7 : 8-*dimethoxy*-1 : 3 : 10-*trimethyl*-2 : 9-*diazaphenanthrene***, prepared by methylating the foregoing compound (1·5 g.) in water (20 ml.) and potassium hydroxide (280 mg.) with methyl sulphate (630 mg.), formed octahedra (from aqueous methanol), m. p. 136—137° (Found: C, 70·3; H, 6·0; N, 8·8. $C_{19}H_{20}O_3N_2$ requires C, 70·6; H, 6·2; N, 8·7%).

5-*Acetyl*-3-*carbethoxy*-1 : 4-*dihydro*-2 : 6-*dimethyl*-4-*o*-*nitrophenylpyridine*, prepared (42%) by heating ethyl β-aminocrotonate (4·5 g.) with *o*-nitrobenzylideneacetylacetone (6·9 g.) for 2 days on the water-bath, formed yellow phototropic prisms from aqueous ethanol; it had m. p. 175·5—176·5° (Found: C, 62·8; H, 5·8; N, 7·8. $C_{18}H_{20}O_5N_2$ requires C, 62·8; H, 5·8; N, 8·1%). The corresponding ***pyridine***, prepared by oxidation of the dihydropyridine with chromium trioxide, followed by reduction of the resulting product with reduced iron, was obtained in faintly green small crystals, m. p. 152—153°, from chloroform–light petroleum (Found: C, 69·4; H, 5·9; N, 9·0. $C_{18}H_{20}O_3N_2$ requires C, 69·5; H, 6·4; N, 9·0%). An acidified solution of this compound, on treatment with nitrous acid at 0°, did not couple immediately with alkaline β-naphthol, but slowly developed a red colour.

10-*Acetoxy*-4-*acetyl*-1 : 3-*dimethyl*-2 : 9-*diazaphenanthrene*, prepared (81%) by heating the foregoing compound (2 g.) with acetic anhydride (10 ml.) under reflux for 3 minutes and pouring on crushed ice (50 g.), formed plates, m. p. 134°, from ethanol or benzene (Found: C, 68·3; H, 5·8; N, 8·2. $C_{17}H_{16}O_3N_2,\frac{1}{2}H_2O$ requires C, 68·1; H, 5·4; N, 8·8%).

5-*Acetyl*-3-*cyano*-1 : 4-*dihydro*-2 : 6-*dimethyl*-4-*o*-*nitrophenylpyridine*, prepared from β-aminocrotononitrile (4·1 g.) and *o*-nitrobenzylideneacetylacetone (11·5 g.), separated from aqueous acetic acid in phototropic golden needles, m. p. 213—214° (Found: C, 63·4; H, 5·3; N, 14·7. $C_{16}H_{15}O_3M_3$ requires C, 64·7; H, 5·1; N, 14·1%). The corresponding ***pyridine*** formed rhombic crystals (from methanol), m. p. 117·5—118·5° (Found: C, 65·3; H, 4·3; N, 14·5. $C_{16}H_{13}O_3N_3$ requires C, 65·1; H, 4·4; N, 14·2%).

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2. *Synthetic Analgesics and Related Compounds. Part III.* The Aminoalkylation of Phenyl-substituted Acetones.*

By WALTER WILSON.

Several γ-dialkylamino-ketones, which had appreciable local anæsthetic activity, have been obtained from phenylacetone or 1 : 3-diphenylacetone by reaction with 2-dialkylaminoethyl chlorides and sodamide in benzene. Enol ethers were not detected in the products. A by-product from 1 : 3-diphenylacetone was the symmetrical bis-2-dimethylaminoethyl derivative (III). The alternative unsymmetrical structure (IV; R = CO·CH_2Ph) was excluded by comparison with the authentic compound of the latter structure, made from 3-cyano-1 : 5-bisdimethylamino-3-phenylpentane (IV; R = CN) and benzylmagnesium chloride. The known analgesic, 5-dimethylamino-3 : 3-diphenylpentan-2-one (VI), was obtained in low yield from 1 : 1-diphenylacetone and 2-dimethylaminoethyl chloride.

A POSSIBLE convenient source of γ-dialkylamino-ketones related to amidone (I) is the condensation between suitable α-phenyl-substituted ketones and 2-dialkylaminoalkyl halides. *C*-Alkylated products (II; R = Me or CH_2Ph) of the required structures have now been obtained from phenylacetone and from 1 : 3-diphenylacetone by treating it with 2-dimethylamino- and 2-diethylamino-ethyl chloride and sodamide in benzene. One of these products (II; R = Me, R′ = Et) has been described previously (Brown, Cook, and Heilbron, *J.*, 1949, S 106).

The bases were unchanged on prolonged boiling with concentrated hydrochloric acid, and they afforded highly crystalline homogeneous hydrogen oxalates. The bases are believed to be substantially free from enol ethers, which, if formed, had been hydrolysed during the subsequent operations. Basic ketones and enol ethers are both obtained in the related reaction of deoxybenzoin with 2-dialkylaminoethyl chlorides (Sperber, Fricano, and Papa, *J. Amer. Chem. Soc.*, 1950, **72**, 3068; Matti and Reynaud, *Bull. Soc. chim.*, 1951, **18**, 31). A secondary product from 1 : 3-diphenylacetone and 2-dimethylaminoethyl chloride was the symmetrical bis-2-dimethylaminoethyl derivative (III) : an enol ether structure was excluded by the stability of the compound to boiling concentrated hydrochloric acid, and the compound was not identical with the unsymmetrical alternative isomer (IV; R = CO·CH_2Ph) made by an unequivocal method from the cyanide (IV; R = CN) and benzylmagnesium chloride. The cyanides (V; R′ = Me or Et) are ketonised by phenylmagnesium bromide (Sperber, Fricano, and Papa, Matti; and Reynaud, *locc. cit.*), but not by methylmagnesium iodide (Brown, Cook, and Heilbron, *loc. cit.*). The latter reaction would provide an alternative route to some of the ketones prepared here, but its failure has been confirmed.

1 : 1-Diphenylacetone reacted only slowly with 2-dimethylaminoethyl chloride, and 73% of the ketone was recovered. The basic condensation product was a mixture, from which 5-dimethylamino-3 : 3-diphenylpentan-2-one (VI) was isolated as the hydrochloride

$Ph_2C(CO\cdot CH_2\cdot CH_3)\cdot CH_2\cdot CHMe\cdot NMe_2$ (I)

$Ph\cdot CH(COR)\cdot CH_2\cdot CH_2\cdot NR'_2$ (II)

$CO(CHPh\cdot CH_2\cdot CH_2\cdot NMe_2)_2$ (III)

$Ph\cdot CR(CH_2\cdot CH_2\cdot NMe_2)_2$ (IV)

$Ph\cdot CH(CN)\cdot CH_2\cdot CH_2\cdot NR'_2$ (V)

$Ph_2C(COMe)\cdot CH_2\cdot CH_2\cdot NMe_2$ (VI)

in only 3% overall yield. Tiffeneau and Levy (*Bull. Soc. chim.*, 1923, **33**, 776) also found that simple alkylations of 1 : 1-diphenylacetone occurred at position 1. Some 1 : 1-diphenylbutan-2-one was made by a new method from diphenylacetyl chloride and diethylcadmium, but it was decided not to treat this with 2-chloro-1-dimethylaminopropane

* Part II, *J.*, 1951, 1706.

because it had become clear that only a minute yield of amidone could be expected, and because Walton, Ofner, and Thorpe (*J.*, 1949, 649) reported the failure of preliminary experiments to make amidone by this method.

Biological examination of the new basic ketones has so far revealed only local anæsthetic properties. The compound (II; R = CH_2Ph, R′ = Me) possessed about 0·4 times the activity of cinchocaine ("Nupercaine") on comparison by Bülbring and Wajda's method (*J. Pharmacol. Exper. Therap.*, 1945, **85**, 78).

Experimental

5-Dimethylamino-3-phenylpentan-2-one (II; R = R′ = Me).—Phenylacetone (26·8 g.), powdered sodamide (8·6 g.), and pure benzene (125 c.c.) were stirred at 25—30° for 15 minutes, then dry 2-dimethylaminoethyl chloride (21·5 g.) in benzene (25 c.c.) was added. On heating to 55°, a vigorous reaction commenced and was completed by stirring at 75—80° for 2 hours. The mixture was washed with water and extracted with 4N-hydrochloric acid. Phenylacetone (6·6 g., 25%) was recovered from the benzene phase, and an oily base (27·9 g.) from the acid extracts. Distillation afforded the *dimethylamino*-compound (23·2 g., 57%), b. p. 96°/1·4 mm., n_D^{20} 1·5040 (Found: C, 76·9; H, 9·2. $C_{13}H_{19}ON$ requires C, 76·1; H, 9·3%). The iodoform test was positive. The hydrochloride and picrate were not obtained crystalline. The base (2·05 g.), ethanol (5 c.c.), and oxalic acid dihydrate (1·3 g.; as a concentrated aqueous solution) afforded the *hydrogen oxalate* (1·25 g.), which formed white needles (from ethanol–acetone), m. p. 122·5° (Found: C, 61·35; H, 6·7. $C_{15}H_{21}O_5N$ requires C, 61·05; H, 7·1%). The base was unchanged after 6 hours in boiling concentrated hydrochloric acid, and the yield of hydrogen oxalate was not affected by this treatment. The base (4·1 g.), hydroxylamine hydrochloride (1·4 g.), acetic acid (0·3 c.c.), and ethanol (25 c.c.) were boiled for 6 hours. Evaporation and addition of ether afforded a solid (5·1 g.; m. p. 169—172°) which was recrystallised from *iso*propanol–ethyl acetate; the *oxime hydrochloride* had m. p. 173° (Found: C, 60·3; H, 8·35. $C_{13}H_{21}ON_2Cl$ requires C, 60·8; H, 8·2%).

5-Diethylamino-3-phenylpentan-2-one (II; R = Me, R′ = Et).—A similar reaction using phenylacetone and 2-diethylaminoethyl chloride afforded the diethylamino-compound, b. p. 134°/3 mm., n_D^{20} 1·4992, in 54% yield (Brown, Cook, and Heilbron, *loc. cit.*, give yield 43%, b. p. 102—105°/0·1 mm., n_D^{24} 1·4958). The iodoform test was positive, and the *hydrogen oxalate* was obtained in 77% yield in aqueous ethanol–ether and formed prisms, m. p. 125° (Found: N, 4·4. $C_{17}H_{25}O_5N$ requires N, 4·35%).

Reaction of 1 : 3-*Diphenylacetone with* 2-*Dimethylaminoethyl Chloride*.—Redistilled 1 : 3-diphenylacetone (26·3 g.), powdered sodamide (5·5 g.), and dry benzene (60 c.c.) were stirred for 30 minutes with cooling to below 30°. 2-Dimethylaminoethyl chloride (anhydrous) (13·4 g.) in benzene (15 c.c.) was added and the mixture warmed to 65—70°. When the vigorous reaction subsided, the mixture was stirred under reflux for 2¼ hours. Extraction with dilute hydrochloric acid afforded a neutral and a basic fraction. The neutral fraction (10·5 g.) was mainly 1 : 3-diphenylacetone, but crystals of impure stilbene (an artefact?) (0·5 g.), m. p. 115·5—117·5°, slowly separated (Found: C, 93·6; H, 7·0. Calc. for $C_{14}H_{12}$: C, 93·3; H, 6·7%); a mixture with authentic stilbene (m. p. 123°) had m. p. 117—122°. The solution of the basic fraction in dilute hydrochloric acid was evaporated to dryness and the solid extracted with hot absolute ethanol. Recrystallisation of the sparingly soluble portion from aqueous ethanol–acetone afforded 1 : 7-*bisdimethylamino*-3 : 5-*diphenylheptan*-4-*one dihydrochloride* (cf. III) (2·2 g.) as small prisms, m. p. 270° (decomp.) (Found: C, 65·0; H, 8·1. $C_{23}H_{32}ON_2,2HCl$ requires C, 64·95; H, 8·0%). The corresponding *dipicrate* formed bright yellow prisms, m. p. 196—197°, from aqueous acetone–ether (Found: N, 13·8. $C_{35}H_{38}O_{15}N_8$ requires N, 13·85%). The more soluble portion of the crude hydrochloride yielded 5-*dimethylamino*-1 : 3-*diphenylpentan*-2-*one hydrochloride* (10·6 g.), m. p. 149·5—151°, on crystallisation from *iso*propanol–ether (Found: C, 71·95; H, 7·7. $C_{19}H_{23}ON,HCl$ requires C, 71·8; H, 7·55%).

5-*Diethylamino*-1 : 3-*diphenylpentan*-2-*one* (II; R = CH_2Ph, R′ = Et).—A similar reaction between 1 : 3-diphenylacetone (17·4 g.), sodamide (3·5 g.), and 2-diethylaminoethyl chloride (11·2 g.) in benzene (55 c.c.) afforded a neutral fraction (11·2 g.) which was mainly 1 : 3-diphenylacetone, and a basic fraction. The latter was refluxed for 4 hours with concentrated hydrochloric acid, then isolated and distilled, yieding the 5-*diethylamino*-compound (9·0 g.), b. p. 152—154°/0·04 mm., n_D^{25} 1·5382 (Found: C, 81·55; H, 9·05. $C_{21}H_{27}ON$ requires C, 81·55; H, 8·75%). The hydrochloride was not obtained crystalline.

3-*Cyano*-1 : 5-*bisdimethylamino*-3-*phenylpentane* (IV; R = CN).—1-Cyano-3-dimethylamino-1-phenylpropane (9·4 g.) was stirred at 25—35° for 10 minutes with powdered sodamide (2·5 g.) in benzene (25 c.c.). 2-Dimethylaminoethyl chloride (anhydrous) (5·4 g.) in benzene (12 c.c.) was added and the reaction completed by boiling for 2 hours. The resulting *cyano*-base (9·4 g., 72·5%) had b. p. 116—120°/0·02 mm., n_D^{22} 1·5088 (Found: N, 15·9. $C_{16}H_{25}N_3$ requires N, 16·2%). The carbonate was formed as a white solid on exposure to air, and the *dihydrochloride* was precipitated as a gelatinous solid on addition of ether to a solution in ethanol. After being dried at 100°/12 mm., it had m. p. 272·5—273° (Found: N, 12·65. $C_{16}H_{25}N_3,2HCl$ requires N, 12·65%).

5-*Dimethylamino*-3-2′-*dimethylaminoethyl*-1 : 3-*diphenylpentan*-2-*one* (IV; R = ·CO·CH_2Ph).—The above cyanide (6·4 g.) in benzene (35 c.c.) was mixed with a Grignard solution made from benzyl chloride (10·2 g.), magnesium (2·1 g.), and ether (25 c.c.). The ether was distilled off and the residual solution vigorously refluxed for 22 hours. Excess of dilute hydrochloric acid was added and the mixture boiled for 3 hours. The benzene layer was removed and excess of ammonia added to the aqueous layer. The precipitated *base* (8·6 g., 99%), isolated by extraction with benzene and distillation, had b. p. 160°/0·01 mm., n_D^{25} 1·5444 (Found: N, 7·75. $C_{23}H_{32}ON_2$ requires N, 7·95%). The *dipicrate* formed a bright yellow crystalline powder, m. p. 175—176°, from acetone–ethyl acetate (Found: N, 14·05. $C_{35}H_{38}O_{15}N_8$ requires N, 13·85%). The dihydrochloride was not obtained crystalline.

Hydrogen Oxalates of **3-*Dialkylamino*-1-*phenylpropyl Cyanides***.—The 3-dimethylamino-compound (1·9 g.) and oxalic acid dihydrate (1·3 g.) in aqueous ethanol afforded the *hydrogen oxalate* (1·8 g.), m. p. 153° (Found: C, 60·4; H, 6·7. $C_{14}H_{18}O_4N_2$ requires C, 60·45; H, 6·5%). The 3-diethylamino-compound (2·2 g.) similarly afforded the *hydrogen oxalate* (2·3 g.), m. p. 168—171°, which formed flakes from ethanol–acetone (Found: C, 62·8; H, 7·2. $C_{16}H_{22}O_4N_2$ requires C, 62·75; H, 7·2%).

Attempted Ketonisation of **3-*Dialkylamino*-1-*phenylpropyl Cyanides***.—The 3-dimethylamino-compound (9·4 g.) in benzene (24 c.c.) was vigorously boiled for 4 hours with a Grignard solution made from methyl iodide (15·6 g.), magnesium (2·9 g.), and ether (20 c.c.). The basic product was unchanged cyanide (7·25 g., 71%), b. p. 98—103°/0·5 mm., n_D^{20} 1·5081, identified by conversion into the hydrogen oxalate, m. p. 153° (4·55 g. from 5·6 g. of base). In a similar experiment, 3-diethylamino-1-phenylpropyl cyanide was recovered unchanged in 68% yield, and there was again no evidence of ketonisation.

Reaction of **1 : 1-*Diphenylacetone* with 2-*Dimethylaminoethyl Chloride***.—The ketone (10·5 g.), dry benzene (30 c.c.), and sodamide (2·5 g.) were stirred at 35—45° for 30 minutes. 2-Dimethylaminoethyl chloride (5·4 g.) in benzene (10 c.c.) was added and the mixture heated slowly to boiling. After 2 hours' refluxing, the mixture was extracted with dilute hydrochloric acid to afford unchanged 1 : 1-diphenylacetone (7·7 g., 73%), m. p. 58·5—60·5°, and a basic fraction (1·8 g.), b. p. 136—145°/0·15 mm. The latter gave a gummy hydrochloride which was repeatedly recrystallised from *iso*propanol–ether, to give 5-dimethylamino-3 : 3-diphenylpentan-2-one hydrochloride (0·45 g., 3%), m. p. 182—184°, not depressed on admixture with an authentic specimen (following experiment). The hydrochloride (0·25 g.) was converted into the free base and added to toluene-*p*-sulphonic acid (0·15 g.) in *iso*propanol ether. The resulting *toluene-p-sulphonate* (0·2 g.) formed solvated prisms, m. p. 89—90°, which had m. p. 109·5—110° after being dried at 80°/15 mm. (Found: N, 2·9. $C_{26}H_{31}O_4NS$ requires N, 3·8%). This was identical with the toluene-*p*-sulphonate prepared in the following experiment.

5-*Dimethylamino*-3 : 3-*diphenylpentan*-2-*one* (VI).—The base was prepared from the corresponding cyanide and methylmagnesium iodide (Dupré, Elks, Hems, Speyer, and Evans, *J.*, 1949, 510). The hydrochloride formed white prisms, m. p. 182·5—184° (Dupré *et al.* give m. p. 186—187·5°; Walton, Ofner, and Thorpe, *J.*, 1949, 654, give m. p. 152—153°). The toluene-*p*-sulphonate (0·45 g. from 0·4 g. of base) formed solvated prisms, m. p. 90·5—92°, from acetone–ether.

1 : 1-*Diphenylbutan*-2-*one*.—Diphenylacetic acid (106 g.) and thionyl chloride (43 c.c.) were refluxed for 4 hours. The fraction of b. p. 158—170°/4·5 mm. (101 g., 88%) gave pure diphenylacetyl chloride, m. p. 54—56°, on trituration with light petroleum (b. p. 40—60°). The acid chloride (18·4 g.) in benzene (115 c.c.) was added to a solution of diethylcadmium prepared in the usual way from ethyl bromide (10·9 g.), magnesium (2·7 g.), ether (100 c.c.), and anhydrous cadmium chloride (9·2 g.). The ether was distilled off and the residual benzene suspension refluxed for 3 hours. The mixture was decomposed with dilute hydrochloric acid and fractionated to yield 1 : 1-diphenylbutan-2-one (9·8 g., 56%), b. p. 175—180°/5 mm. (semicarbazone, m. p. 185°), and (non-volatile) diphenylacetic anhydride (6·25 g., 39%), colourless

needles, m. p. 100° (from methanol), converted by aqueous ammonia into diphenylacetamide, m. p. 168·5—169·5°.

The author is indebted to Professor M. Stacey, F.R.S., for encouragement and for facilities and to Mr. B. Stringer for the microanalyses. He also thanks Dr. P. B. Marshall, Mr. C. G. Haining, and Dr. Nazeer ud din Ahmad of the Department of Pharmocology of this University for bioassays, full details of which will be published elsewhere.

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EDGBASTON, BIRMINGHAM, 15. [*Received, September 19th*, 1951.]

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3. 5-*Hydroxy*-1 : 2-*benzanthracene and* 1′-*Hydroxy*-1 : 2-5 : 6-*dibenzanthracene.*

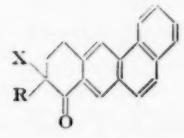
By J. W. COOK and (MISS) R. SCHOENTAL.

1′ Hydroxy-1 : 2-5 : 6-dibenzanthracene has been synthesised from 5 : 6 : 7 : 8-tetrahydro-5-keto-1 : 2-benzanthracene, which has also been dehydrogenated to 5-hydroxy-1 : 2-benzanthracene. The dibenzanthracene derivative was different from a metabolite of the related hydrocarbon; the 5-hydroxybenzanthracene was required for comparison of the carcinogenic properties of its methyl ether with those other 5-substituted 1 : 2-benzanthracenes.

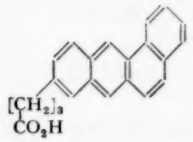
THE carcinogenic hydrocarbon 1 : 2-5 : 6-dibenzanthracene is metabolised by rats and mice to the 4′ : 4″-dihydroxy-derivative, and by rabbits to an isomeric dihydroxy-compound of unknown orientation (for references, see Williams, "Detoxication Mechanisms," London, 1947, p. 51). We have re-examined the metabolism of 1 : 2-5 : 6-dibenzanthracene in rabbits, using the procedure described by Berenblum and Schoental (*Cancer Research*, 1943, **3**, 145) for analogous cases. The benzene and chloroform extracts of the dried fæces, excreted after intraperitoneal injection of a suspension of dibenzanthracene in tricaprylin, were adsorbed on a column of alumina, and the material from the fluorescent zone was treated with methyl sulphate and sodium hydroxide solution at 100°. Further chromatographic purification then led to two crystalline products. One of these crystallised from benzene in almost colourless needles, m. p. 244—245°, and was probably the dimethoxy-1 : 2-5 : 6-dibenzanthracene isolated from the methylated phenols from the urine of rabbits dosed with 1 : 2-5 : 6-dibenzanthracene by Boyland, Levy, Mawson, and Roe (*Biochem. J.*, 1941, **35**, 184), whose product had this m. p. A less strongly adsorbed fluorescent substance crystallised from methanol in fine colourless needles, m. p. 214°. The band of shortest wave-length in its fluorescent spectrum had its maximum intensity at 4005 Å, compared with a value of 4070 Å for the dimethoxydibenzanthracene. These relations are consistent with the view that the new methoxy-compound is a monomethoxydibenzanthracene. 9-Methoxy-1 : 2-5 : 6-dibenzanthracene gave a value of 3995 Å for the maximum of its corresponding fluorescence band, and 3-methoxy-1 : 2-5 : 6-dibenzanthracene a value of 4027 Å. The non-identity of the new methylated metabolite with either of these methoxydibenzanthracenes was confirmed by mixed m. p. determinations.

Although 55 isomeric dimethoxy-1 : 2-5 : 6-dibenzanthracenes are possible, the number of possible monomethoxy-compounds is only 7. Two of these have been excluded by the comparisons just mentioned. Moreover, all the identified phenolic metabolic products of fused-ring aromatic hydrocarbons have hydroxyl groups in α-positions to points of ring-fusion. Consequently, if the new compound is, as suggested, a monomethoxydibenzanthracene, the substituent is unlikely to be in the β-positions 2′ or 3′, and we are left with three positions for consideration (1′, 4′, and 4). The synthesis of all three seemed feasible, and the present communication deals with the synthesis of 1′-hydroxy-1 : 2-5 : 6-dibenzanthracene and its methyl ether, which proved to be different from the methylated metabolite, m. p. 214°.

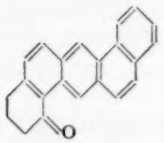
The starting point in our synthesis was 5 : 6 : 7 : 8-tetrahydro-5-keto-1 : 2-benzanthracene (I; R = X = H), the 6-carbomethoxy-derivative of which was condensed with γ-iodobutyronitrile to give, after hydrolysis and dehydrogenation, γ-(1 : 2-benzanthr-6-yl)butyric acid (II). This was cyclised to 1′ : 2′ : 3′ : 4′-tetrahydro-1′-keto-1 : 2-5 : 6-dibenzanthracene (III), from which 1′-hydroxy-1 : 2-5 : 6-dibenzanthracene was obtained by treatment with palladium black in boiling 1-methylnaphthalene.



(I)



(II)



(III)

The very high carcinogenic potency of 8-methoxy-3 : 4-benzpyrene (Cook, Ludwiczak, and Schoental, *J.*, 1950, 1112) made it of interest to compare the biological properties of 5-methoxy-1 : 2-benzanthracene with those of other 5-substituted 1 : 2-benzanthracenes, several of which are carcinogenic. To this end, 5 : 6 : 7 : 8-tetrahydro-5-keto-1 : 2-benzanthracene was dehydrogenated to 5-hydroxy-1 : 2-benzanthracene, which was then methylated.

Experimental

Methyl 6-3′-*Cyanopropyl*-5 : 6 : 7 : 8-*tetrahydro*-5-*keto*-1 : 2-*benzanthracene*-6-*carboxylate* (I; R = $[CH_2]_3$·CN, X = CO_2Me).—Methyl 5 : 6 : 7 : 8-tetrahydro-5-keto-1 : 2-benzanthracene-6-carboxylate (Bachmann and Chemerda, *J. Org. Chem.*, 1941, **6**, 47) (5 g.) was added to sodium methoxide solution prepared from sodium (0·8 g.) and methanol (10 c.c.) in benzene (50 c.c.), and the suspension heated on the water-bath for ½ hour. γ-Iodobutyronitrile (Cook and Lawrence, *J.*, 1937, 821) (10 c.c.) was then added and heating continued for 18 hours. A further quantity (5 c.c.) of the iodo-nitrile was added, and heating continued for a further 24 hours. Water was then added to the cooled suspension, and the oil which separated was extracted with chloroform. The concentrated extract gave a solid which was further purified by passage of its benzene solution through a column of alumina. The resulting *keto*-ester (I; R = $[CH_2]_3$·CN; X = CO_2Me) formed colourless silky needles (from ethyl acetate), m. p. 178—179° (Found: C, 77·3; H, 6·0; N, 3·55. $C_{24}H_{21}O_3N$ requires C, 77·6; H, 5·7; N, 3·8%).

γ-(5 : 6 : 7 : 8-*Tetrahydro*-5-*keto*-1 : 2-*benzanthr*-6-*yl*)*butyric Acid* (I; R = $[CH_2]_3$·CO_2H, X = H).—A solution of the aforesaid keto-ester (3 g.) in acetic acid (30 c.c.) and concentrated hydrochloric acid (30 c.c.) was boiled under reflux for 3 hours. More hydrochloric acid (10 c.c.) was added and boiling continued for 2 hours. The solid which separated on cooling (1 g.) was collected and recrystallised from acetic acid (charcoal). The substituted *butyric acid* (I; R = $[CH_2]_3$·CO_2H; X = H) formed colourless leaflets, m. p. 191—192° (Found: C, 79·6; H, 5·9. $C_{22}H_{20}O_3$ requires C, 79·5; H, 6·1%). Its *methyl* ester formed thin elongated crystals (from methanol), m. p. 124—125° (Found: C, 79·8; H, 6·3; OMe, 9·3. $C_{23}H_{22}O_3$ requires C, 79·7; H, 6·4; OMe, 9·0%).

γ-(5 : 6 : 7 : 8-*Tetrahydro*-1 : 2-*benzanthr*-6-*yl*)*butyric Acid.*—A solution of the keto-acid (1 g.) in diethylene glycol (10 c.c.) containing potassium hydroxide (0·8 g.) was reduced with hydrazine hydrate (1 c.c.), following the procedure of Huang-Minlon (*J. Amer. Chem. Soc.*, 1946, **68**, 2487). The reduced *acid* formed colourless leaflets (from ethyl acetate), m. p. 175·5—176·5° (Found: C, 82·9; H, 7·1. $C_{22}H_{22}O_2$ requires C, 82·95; H, 7·0%), and gave a *methyl* ester, m. p. 90—91·5° (Found: C, 83·3; H, 7·35. $C_{23}H_{24}O_2$ requires C, 83·1; H, 7·3%).

γ-(1 : 2-*Benzanthr*-6-*yl*)*butyric Acid* (II).—The aforesaid ester (1 g.) was heated with palladium black (100 mg.) in a nitrogen atmosphere at 280° for 3 hours. The product was hydrolysed with methanolic potassium hydroxide and separated into neutral and acidic components. The neutral material, purified by passage of its solution in benzene–light petroleum through alumina, was evidently 6-n-*propyl*-1 : 2-*benzanthracene*, which formed colourless leaflets (from methanol), m. p. 129° after softening (Found: C, 92·8; H, 7·3. $C_{21}H_{18}$ requires C, 93·3; H, 6·7%). Its deep orange *s-trinitrobenzene* complex had m. p. 127—128° (Found: C, 66·9; H, 4·6; N, 9·0. $C_{27}H_{21}O_6N_3$ requires C, 67·1; H, 4·4; N, 8·7%).

The acidic fraction from the dehydrogenation yielded γ-(1 : 2-*benzanthr*-6-*yl*)*butyric acid*

(II) as colourless leaflets (from benzene), m. p. 192—193° (Found : C, 83·9; H, 5·8. $C_{22}H_{18}O_2$ requires C, 84·0; H, 5·8%).

1′ : 2′ : 3′ : 4′-Tetrahydro-1′-keto-1 : 2-5 : 6-dibenzanthracene (III).—A solution of the acid (II) (150 mg.) in benzene (5 c.c.) was treated with phosphorus pentachloride (0·2 g.) and kept at room temperature for 2 hours. Anhydrous stannic chloride (0·2 c.c.) was then added to the ice-cold solution, which was kept at 0° for ½ hour and then treated with ice and hydrochloric acid. The resulting *1′ : 2′ : 3′ : 4′-tetrahydro-1′-keto-1 : 2-5 : 6-dibenzanthracene* (III), recovered from an ethereal extract which had been washed with sodium carbonate solution, formed rhombic yellowish crystals (from benzene), m. p. 168—169° (Found : C, 89·2; H, 5·1. $C_{22}H_{16}O$ requires C, 89·1; H, 5·4%).

1′-Hydroxy-1 : 2 - 5 : 6-dibenzanthracene.—A solution of the pentacyclic ketone (60 mg.) in 1-methylnaphthalene (3 c.c.) was boiled in a nitrogen atmosphere with palladium black (10 mg.) for 12 hours. Benzene was added to the cooled solution. *1′-Hydroxy-1 : 2-5 : 6-dibenzanthracene* crystallised and after recrystallisation from benzene formed pale yellow threads, m. p., in an evacuated sealed capillary, 269—270° (decomp.) (Found : C, 89·8; H, 5·0. $C_{22}H_{14}O$ requires C, 89·8; H, 4·8%). Its *methyl ether*, prepared by treatment with methyl sulphate and sodium hydroxide solution and purified by chromatography on alumina, formed colourless needles (from benzene–methanol), micro-m. p. 157—160° (Found : C, 89·2; H, 5·6. $C_{23}H_{16}O$ requires C, 89·5; H, 5·2%).

The ultra-violet absorption spectrum of this methoxy-compound closely resembled that of 1 : 2-5 : 6-dibenzanthracene. The wave-lengths and extinction coefficients of the maxima were as follows : λ 400, 380, 350, 335, 325, 294, 280 mμ.; log ε 3·25, 3·30, 4·12, 4·32, 4·49, 5·06, 4·78.

5-Hydroxy-1 : 2-benzanthracene.—A solution of 5 : 6 : 7 : 8-tetrahydro-5-keto-1 : 2-benzanthracene (I; R = X = H) (Haworth and Mavin, *J.*, 1933, 1012) (1 g.) in 1-methylnaphthalene (10 c.c.) was boiled in a nitrogen atmosphere with palladium black (100 mg.) for 12 hours. The crystals which separated on cooling were recrystallised from benzene and gave *5-hydroxy-1 : 2-benzanthracene* as yellowish silky threads, m. p. 217—218°, in an evacuated sealed capillary (Found : C, 88·3; H, 5·2. $C_{18}H_{12}O$ requires C, 88·5; H, 5·0%). Its *methyl ether* formed colourless needles (from benzene–light petroleum), m. p. 186—187° (Found : C, 88·4; H, 5·6. $C_{19}H_{14}O$ requires C, 88·3; H, 5·5%), and gave a crimson *s-trinitrobenzene* complex, m. p. 197—198° (decomp.) (Found : C, 63·6; H, 4·0; N, 9·3. $C_{25}H_{17}O_7N_3$ requires C, 63·7; H, 3·6; N, 8·9%).

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UNIVERSITY OF GLASGOW. [*Received, September 20th*, 1951.]

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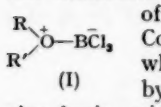
4. *The Compound of Boron Trichloride with Dioxan.*

By A. K. HOLLIDAY and JOAN SOWLER.

Boron trichloride reacts with dioxan at 20° to give an equimolecular compound $BCl_3,C_4H_8O_2$. The compound $2BCl_3,C_4H_8O_2$ cannot be prepared by allowing an excess of boron trichloride to react with either dioxan or the 1 : 1-compound.

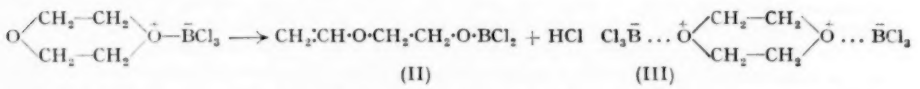
FEW compounds in which boron trichloride is directly co-ordinated to a carbon-linked oxygen atom have been isolated. The formation of compounds $>CO,BCl_3$ has been assumed in some cases, especially if the corresponding trifluoride has been identified, but immediate breakdown occurs with evolution of hydrogen chloride and formation of complex mixtures of products (Martin, *Chem. Reviews*, 1944, **34**, 468). Co-ordination compounds of the type (I) appear to be rather more stable when R = R′ = Me or Et; *e.g.*, boron trichloride–dimethyl ether is formed by direct reaction at −80° but decomposes at higher temperatures to give boric oxide, methyl chloride, and methyl dichloroborate (Wiberg and Sütterlin, *Z. anorg. Chem.*, 1931, **202**, 1, 22, 31, 37). The diethyl ether compound (I; R = R′ = Et) is also prepared by direct reaction and is relatively stable in dry air



(I)

(Nespital, *Z. physikal. Chem.*, 1932, *B*, **16**, 173). When R′ = H, isolation of compounds ROH,BCl_3 has not been possible, because these lose hydrogen chloride to yield successively the esters $BCl_2{\cdot}OR$, $BCl(OR)_2$, and $B(OR)_3$ (R = Me, Et) (Wiberg and Sütterlin, *loc. cit.*; Wiberg and Smedsund, *Z. anorg. Chem.*, 1935, **225**, 204; Wiberg and Ruschmann, *Ber.*, 1937, **70**, 1393).

Lane, McCusker, and Curran (*J. Amer. Chem. Soc.*, 1942, **64**, 2076) measured electric moments of boron trichloride in dioxan as solvent. Comparison of the observed dipole moment with that of (I; R = R′ = Et) suggested that co-ordination to give a 1 : 1-compound was almost complete in solution, but the compound was not isolated. We have found that slow reaction of boron trichloride with dioxan in a vacuum at 20° yields colourless hexagonal crystals with composition and molecular weight corresponding to $C_4H_8O_2,BCl_3$. This substance is stable in dry air, but reacts instantaneously with water to yield boric acid; it is soluble in dioxan and in dibenzyl ether, sparingly soluble in benzene and carbon tetrachloride, and insoluble in *cyclo*hexane. When heated *in vacuo*, it darkens, and becomes blue at 75°. At 78·5°, it melts to a deep blue liquid and this change is accompanied by an abrupt discontinuity in the vapour pressure–temperature curve. Further heating at 78·5° causes an increase in vapour pressure; when the system is cooled and the products fractionated, there remains a non-volatile tarry solid containing boron and chlorine. The volatile products are hydrogen chloride and a viscous liquid which finally crystallises to give a white solid. The latter reacts with moist air, evolving hydrogen chloride; it attacks tap grease readily and is rather unstable. Analysis for boron and chlorine gives values in agreement with those required for 2-vinyloxyethyl dichloroborate (II), but the substance is too unstable to permit determination of its molecular weight. The thermal decomposition of boron trichloride–dioxan can then be represented as



(II) (III)

the breakdown being similar to that observed with boron trichloride–ether compounds.

Attempts to prepare di(boron trichloride)–dioxan were unsuccessful. Treatment of dioxan with a large excess of boron trichloride, or of boron trichloride–dioxan with boron trichloride at pressures greater than atmospheric and at temperatures up to 70°, yielded only the 1 : 1-compound. If the boron–oxygen link in the 1 : 1-compound is covalent, it is difficult to see how formation of this link can greatly affect the ability of the second dioxan oxygen atom to co-ordinate with another molecule of boron trichloride, especially as the two oxygen atoms are not linked by a conjugated ring or other potentially resonating system. However, Coates (*J.*, 1951, 2003) has suggested that co-ordination compounds of oxygen differ from those of other donor atoms in that the binding is primarily electrostatic rather than covalent, the oxygen being held by attraction between its negatively charged lone pairs and the positive centre of the acceptor. McCusker and Curran (*J. Amer. Chem. Soc.*, 1942, **64**, 614) have noted the relatively small moment of the $\bar{B}$–$\overset{+}{O}$ link (in Et_2O,BCl_3) compared with that of other co-ordinate linkages; this is to be expected if the oxygen–boron linkage is electrostatic rather than covalent. Moreover, compounds of the form A_2D (where A is an acceptor molecule and D is dioxan) have not been isolated, and Lane, McCusker, and Curran (*loc. cit.*) found no evidence for the existence of such compounds in dioxan solutions. Since the formation of one electrostatic oxygen–boron link gives a compound of limited stability, formation of a second link as in (III), involving two positively charged oxygen atoms in the same saturated ring, cannot be expected to occur at a temperature above the m. p. of dioxan.

Experimental

Apparatus and Materials.—A high-vacuum apparatus of conventional type was used in the preparative work, with a vessel through which known volumes of boron trichloride vapour could be passed. Reaction products were fractionated in U-traps on the main vacuum line.

Transfers of moisture-sensitive materials to and from the reaction vessel were made in a dry box. Boron trichloride was purified by fractional condensation in a vacuum apparatus of the Stock type to avoid excessive attack of tap grease; the material used had a vapour pressure of 3—4 mm. at −78°. Dioxan was refluxed over sodium for 6 hours, and the fraction of b. p. 101—101·3°/760 mm. collected.

Reaction of Boron Trichloride and Dioxan.—In a typical experiment, excess of dioxan (60 mmols.) was frozen in the reaction vessel, the apparatus evacuated, and the temperature brought to 20°. Boron trichloride (15 mmols.) was bubbled slowly through the dioxan (rapid bubbling gave a violent reaction). Volatile material was then removed, condensed in liquid nitrogen, and fractionated; a small amount of hydrogen chloride (0·71 mmol.) and dioxan were the only constituents. The remaining *addition compound* was purified by sublimation *in vacuo* below 50°; the yield was 80%.

Analysis for chlorine and boron. Complete hydrolysis of the product without loss of hydrogen chloride was attained either by sealing a weighed sample into a capillary-tipped tube and breaking the capillary under potassium hydroxide solution, or by condensing water on to the sample in an evacuated tube at a low temperature and allowing the whole to warm slowly. Boron was then determined volumetrically in presence of mannitol, and chlorine gravimetrically as silver chloride in solutions sufficiently acid to retain silver borate in solution (Found : Cl, 50·8; B, 5·2. $C_4H_8O_2,BCl_3$ requires Cl, 51·8; B, 5·3%). A specimen exposed to air and allowed to attain constant weight gave Cl, 0; B, 16·5% (Calc. for H_3BO_3 : B, 17·7%).

Molecular weight. A cryoscopic method was used with dioxan as solvent in an apparatus similar to that of Zeffert and Hormatz (*Anal. Chem.*, 1949, **21**, 1420), and adapted to exclude water vapour. Temperatures were measured with a type F2311/300 thermistor (Standard Telephone and Cables, Ltd.), the resistance being determined to ±1 ohm by an ordinary bridge circuit. The apparatus was calibrated by using solutions of naphthalene in dioxan. Resistance of the thermistor at the freezing point of dioxan was 2336Ω, and at this temperature a resistance change of ±1Ω corresponded to ∓0·008°. The cryoscopic constant for dioxan was taken as 4710/100 g. of solvent (Found : *M*, 203 ± 4. $C_4H_8O_2,BCl_3$ requires *M*, 205).

Vapour Pressure and Thermal Decomposition.—Vapour pressure–temperature curves were determined between 17° and 156°, Stock and Küss's isoteniscope method (*Ber.*, 1914, **47**, 3115) being used. Heating and cooling curves did not coincide, and at 78·5° the discontinuity in the v.p.–*T* curve was observed. Prolonged heating at this temperature resulted in a marked increase in vapour pressure, the magnitude depending on the amount of material present. Thermal decomposition was therefore studied by placing the compound in a tube attached to the vacuum line and keeping it at 78·5—82° for about 5 hours; the volatile products were condensed in liquid nitrogen and fractionated. Hydrogen chloride was identified (v.p. at −112°, 122 mm.), and a viscous liquid which gave Cl, 45; B, 6·2% (*2-vinyloxyethyl dichloroborate*, $C_4H_7O_2Cl_2B$, requires Cl, 42·0; B, 6·4%).

UNIVERSITY OF LIVERPOOL. [*Received, September 26th*, 1951.]

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5. 1 : 2-*Dihydro*-2-*thianaphthalene Derivatives. Part II.* Conversion of* 1 : 2-*Dihydro*-1-*keto*-2-*thianaphthalenes into Indanones.*

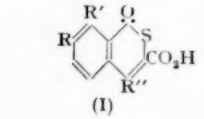
By D. J. DIJKSMAN and G. T. NEWBOLD.

Treatment of 1 : 2-dihydro-1-keto-2-thianaphthalene-3-carboxylic acid and its 7 : 8-dimethoxy- and 4-methyl derivatives with Raney nickel give respectively indan-1-one, 6 : 7-dimethoxyindan-1-one, and 3-methylindan-1-one. The first compound is also obtained by the action of Raney nickel on 1 : 2-dihydro-1-keto-2-thianaphthalene.

IN Part I * of this series we described the preparation of rhodanine condensation products of benzoic acids with an *o*-carbonyl function and their conversion by sodium hydroxide into 1 : 2-dihydro-1-keto-2-thianaphthalene-3-carboxylic acids (I). It was further shown that the sulphur atom in the latter compounds was readily eliminated by ammonia and

* Part I, *J.*, 1951, 1213.

primary amines with the formation of *iso*quinoline derivatives. Continuing our studies on the reactions of thianaphthalenes we have examined their desulphurisation by the action of Raney nickel. Use of this reagent for the formation of aldehydes from thiol-esters, R·CO·SR′ ⟶ R·CHO, has been reported by Wolfrom and Karabinos (*J. Amer. Chem. Soc.*, 1946, **68**, 724, 1455; cf. Spero, McIntosh, and Levin, *ibid.*, 1948, **70**, 1907). Desulphurisation of the thio-lactone acid (I; R = R′ = R″ = H) might therefore have been expected to give *o*-formylcinnamic acid (II). In fact the reaction product was a neutral carbonyl compound, isolated as its semicarbazone and its 2 : 4-dinitrophenylhydrazone which proved to be identical with the corresponding derivatives of indan-1-one (III; R = R′ = R″ = H). Wiley and Hobson (*ibid.*, 1949, **71**, 2429) prepared *o*-formyl-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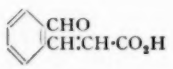
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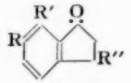
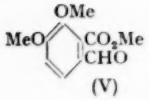

(III)

(IV)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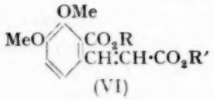
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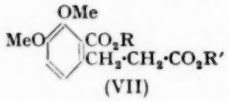
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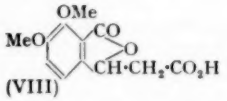
(VIII)

cinnamic acid (II) by the interaction of phthalaldehyde with malonic acid in the presence of pyridine and reported that it lost carbon dioxide readily with the formation of indan-1-one. Reaction of 1 : 2-dihydro-1-keto-2-thianaphthalene (IV) with Raney nickel also gave indan-1-one (III; R = R′ = R″ = H) in fair yield; in this case the intermediate *o*-vinylbenzaldehyde or more probably a related ion cyclises to give (III; R = R′ = R″ = H) by a mechanism similar to that suggested by Wiley and Hobson (*loc. cit.*) for the cyclisation of *o*-formylcinnamic acid; this involves the reaction of the ion formed by decarboxylation of *o*-formylcinnamic acid with the carbonium ion of the carbonyl group rather than with the liberated proton, the latter combining with the carbonyl-oxygen anion. The resulting 1-hydroxyindene is then converted into indan-1-one by a prototropic change.

The general nature of the above desulphurisation reaction has been demonstrated by its application to 1 : 2-dihydro-1-keto-4-methyl- (I; R = R′ = H, R″ = Me) and to 1 : 2-dihydro-1-keto-7 : 8-dimethoxy-2-thianaphthalene-3-carboxylic acid (I; R = R′ = OMe, R″ = H). In the first case the product was 3-methylindan-1-one (III; R = R′ = H, R″ = Me) and, in the second, 6 : 7-dimethoxyindan-1-one (III; R = R′ = OMe, R″ = H), both compounds being isolated as their semicarbazones and 2 : 4-dinitrophenylhydrazones.

6 : 7-Dimethoxyindan-1-one, for comparison with the specimen obtained by the desulphurisation method, was prepared by a modification of the route described by Schöpf, Jackh-Tettweiler, Meyer, Perry-Fehrenbach, and Winterhalter (*Annalen*, 1940, **544**, 77). Condensation of α-methyl opianate (V) (Bain, Perkin, and Robinson, *J.*, 1914, 2392) with malonic acid in pyridine containing piperidine gives 2-carbomethoxy-3 : 4-dimethoxycinnamic acid (VI; R = Me, R′ = H) characterised by the preparation of methyl 2-carbomethoxy-3 : 4-dimethoxycinnamate (VI; R = R′ = Me). Alkaline hydrolysis of (VI; R = Me, R′ = H) gives 2-carboxy-3 : 4-dimethoxycinnamic acid (VI; R = R′ = H) identical with the acid described by Schöpf *et al.* (*loc. cit.*) by the action of alkali on meconin-α-acetic acid (VIII). Crystallisation of 2-carboxy-3 : 4-dimethoxycinnamic acid from dilute hydrochloric acid gives meconin-α-acetic acid; the last compound is also obtained in quantitative yield by fusion of the dicarboxylic acid. Hydrogenation of (VI; R = Me, R′ = H) in the presence of platinum gives β-(2-carbomethoxy-3 : 4-dimethoxyphenyl)propionic acid (VII; R = Me, R′ = H) which on hydrolysis gives β-(2-carboxy-3 : 4-dimethoxyphenyl)propionic acid (VII; R = R′ = H); the latter has been obtained by Schöpf *et al.* (*loc. cit.*) by hydrogenation of 2-carboxy-3 : 4-dimethoxycinnamic acid. Treatment of (VII; R = R′ = H) with acetic anhydride gave 6 : 7-dimethoxyindan-1-one which was identical with the product obtained by desulphurisation of (I; R = R′ = OMe, R″ = H).

Experimental

2-Carbomethoxy-3 : 4-dimethoxycinnamic Acid.—A mixture of α-methyl opianate (10·0 g.), malonic acid (10·0 g.), pyridine (50 c.c.), and piperidine (2 c.c.) was heated for 2 hours on the steam-bath. The cooled reaction mixture was poured into a stirred mixture of ice (150 g.) and hydrochloric acid (100 c.c.; *d* 1·17), and the precipitated solid collected. *2-Carbomethoxy-3 : 4-dimethoxycinnamic acid* (10·0 g., 84%) crystallised from aqueous ethanol as rectangular plates, m. p. 161—162° (Found : C, 59·05; H, 5·7. $C_{13}H_{14}O_6$ requires C, 58·8; H, 5·3%).

Methyl 2-Carbomethoxy-3 : 4-dimethoxycinnamate.—A solution of the acid-ester (2·0 g.) in dry methanol (100 c.c.) containing sulphuric acid (2 c.c.; *d* 1·84) was heated under reflux for 2 hours, and then concentrated under reduced pressure to a bulk of 30 c.c. After the addition of water (200 c.c.) the mixture was extracted with ether (3 × 100 c.c.). The ethereal solution was washed with saturated sodium hydrogen carbonate solution (100 c.c.) and then water (100 c.c.) and dried (Na_2SO_4). The oil (1·7 g.) obtained after removal of the ether crystallised from aqueous methanol to give the *ester* as needles, m. p. 64—66° (Found : C, 59·95; H, 5·8. $C_{14}H_{16}O_6$ requires C, 60·0; H, 5·7%).

2-Carboxy-3 : 4-dimethoxycinnamic Acid.—(*a*) (cf. Schöpf *et al.*, *loc. cit.*). A solution of meconin-α-acetic acid (4·5 g.) in aqueous potassium hydroxide (23 c.c., 50%) was evaporated to dryness on the steam-bath. The residue was dissolved in water (25 c.c.) and the process repeated. Water (50 c.c.) was then added and the solution acidified with hydrochloric acid (2N.) with ice-cooling. The precipitated 2-carboxy-3 : 4-dimethoxycinnamic acid (3·5 g.) separated from water as fine needles, m. p. 171—172° (Schöpf *et al.* record m. p. 178—180°; a mixture with meconinacetic acid had m. p. 158—172°) (Found : C, 57·2; H, 4·9%; equiv., 125. Calc. for $C_{12}H_{12}O_6$: C, 57·1; H, 4·8%; equiv., 126). Light absorption in ethanol : Max. at 2350 (ε = 15,000) and 3000 Å (ε = 18,900).

(*b*) 2-Carbomethoxy-3 : 4-dimethoxycinnamic acid (0·5 g.) in aqueous potassium hydroxide (10 c.c.; 2N.) was heated on the steam-bath for 1 hour. The ice-cooled solution was acidified (Congo-red) with hydrochloric acid (2N.); the precipitate crystallised from water, to give 2-carboxy-3 : 4-dimethoxycinnamic acid (0·35 g.) as fine needles, m. p. 171—172° undepressed by preparation (*a*) (Found : C, 56·6; H, 4·9%; equiv., 130).

Meconin-α-acetic Acid.—(*a*) 2-Carboxy-3 : 4-dimethoxycinnamic acid was melted and then crystallised once from water, to give meconinacetic acid as needles, m. p. 167° (Found : C, 57·3; H, 5·0%; equiv., 265. Calc. for $C_{12}H_{12}O_6$: C, 57·1; H, 4·8%; equiv., 252). Light absorption in ethanol : Max. at 2130 (ε = 25,000) and 3060 Å (ε = 4200).

(*b*) 2-Carboxy-3 : 4-dimethoxycinnamic acid (100 mg.) was crystallised once from hydrochloric acid (6 c.c.; 3N.), to give meconinacetic acid as needles, m. p. 167° (Found : C, 57·35; H, 4·85%; equiv., 248).

β-(2-Carbomethoxy-3 : 4-dimethoxyphenyl)propionic Acid.—2-Carbomethoxy-3 : 4-dimethoxycinnamic acid (1·33 g.) in water (100 c.c.) containing sodium hydrogen carbonate (0·42 g.) was shaken with platinum (from 100 mg. of Adams's platinum oxide) in an atmosphere of hydrogen at 18°/1 atm. When hydrogen absorption ceased (observed, 136 c.c.; calc., 132 c.c.) the catalyst was removed by filtration and the solution acidified (Congo-red) with hydrochloric acid (2N.). The solution was extracted with ether (3 × 40 c.c.), and the dried (Na_2SO_4) extract evaporated to give an oil which rapidly solidified. Crystallisation from light petroleum (b. p. 40—60°) gave *β-(2-carbomethoxy-3 : 4-dimethoxyphenyl)propionic acid* (0·71 g.) as needles, m. p. 88—89° (Found : C, 58·5; H, 6·2. $C_{13}H_{16}O_6$ requires C, 58·3; H, 6·0%). The acid-ester (300 mg.) in potassium hydroxide (5 c.c.; 2N.) was heated on the steam-bath for 1 hour. The solution was acidified (Congo-red) with hydrochloric acid (2N.) and extracted with ether (3 × 10 c.c.), the combined extracts being dried (Na_2SO_4) and evaporated. The residual oil was dissolved in benzene (5 c.c.), and the solvent removed. Crystallisation of the residue from benzene–light petroleum (b. p. 40—60°) gave β-(2-carboxy-3 : 4-dimethoxyphenyl)propionic acid (260 mg.) as small prismatic needles, m. p. 125—126° (Schöpf *et al.*, *loc. cit.*, give m. p. 125—127° for the anhydrous acid prepared by catalytic hydrogenation of 2-carboxy-3 : 4-dimethoxycinnamic acid) (Found : C, 56·5; H, 5·7%; equiv., 125. Calc. for $C_{12}H_{14}O_6$: C, 56·7; H, 5·6%; equiv., 127).

3-Methylindan-1-one.—1 : 2-Dihydro-1-keto-4-methyl-2-thianaphthalene-3-carboxylic acid (Dijksman and Newbold, *loc. cit.*) (0·5 g.) was heated under reflux for 5 hours with a suspension of Raney nickel (5 g.; W.6. prepared according to *Org. Synth.*, **29**, 25) in ethanol (50 c.c.). After removal of the nickel the filtrate and ethanol washings were concentrated to 5 c.c., water (50 c.c.) was added, and the mixture was extracted with ether (4 × 20 c.c.). The ethereal

solution was washed with sodium hydroxide (20 c.c.; N.) and then water (2 × 20 c.c.), and dried (Na_2SO_4). Removal of the ether gave a colourless oil (0·28 g.); treatment of a portion of this oil (0·14 g.) with semicarbazide acetate gave 3-methylindan-1-one semicarbazone (0·16 g.) as needles (from ethanol), m. p. 231° (decomp.) (Found: C, 65·4; H, 6·1. Calc. for $C_{11}H_{13}ON_3$: C, 65·0; H, 6·4%). Light absorption in ethanol: Max. at 2100 (ε = 16,500), 2590 (ε = 17,700), 2790 (ε = 18,400), 2980 (ε = 15,300), and 3080 Å (ε = 13,950); the substance was undepressed in m. p. when mixed with a specimen, m. p. 231° (decomp.) (von Braun and Kirschbaum, *Ber.*, 1913, **46**, 3041, give m. p. 230—231°), prepared from 3-methylindan-1-one obtained by the method of Koelsch, Hochmann, and Le Claire [*J. Amer. Chem. Soc.*, 1943, **65**, 59; absorption max., in ethanol, at 2130 (ε = 15,100), 2690 (ε = 18,100), 2790 (ε = 18,600), 2980 (ε = 15,900), and 3080 Å (ε = 14,600)]. The crude 3-methylindan-1-one (0·14 g.) on treatment with Brady's reagent gave a 2 : 4-dinitrophenylhydrazone (0·26 g.) as red prismatic needles, m. p. 239—240° (decomp.), from ethyl acetate (Found: C, 59·3; H, 3·9. Calc. for $C_{16}H_{14}O_4N_4$: C, 59·3; H, 4·3%). Light absorption in chloroform: Max. at 2500 (ε = 12,000) and 3890 Å (ε = 31,200). It showed no m. p. depression when mixed with an authentic specimen, m. p. 239—240° (decomp.) (Marvel, Dec, and Cooke, *ibid.*, 1940, **62**, 3499, give m. p. 239—241°), which showed absorption max., in chloroform, at 2500 (ε = 11,000) and 3900 Å (ε = 31,000).

6 : 7-*Dimethoxyindan*-1-*one*.—This was obtained as an oil (1·7 g.) by treatment of 1 : 2-dihydro-1-keto-7 : 8-dimethoxy-2-thianaphthalene-3-carboxylic acid (4·0 g.) with Raney nickel as described above. The oil (0·5 g.) on treatment with aqueous-ethanolic semicarbazide acetate gave 6 : 7-dimethoxyindan-1-one semicarbazone (0·32 g.) as small needles, m. p. 217—219° (decomp.), from aqueous ethanol (Found: C, 57·5; H, 6·0. Calc. for $C_{12}H_{15}O_3N_3$: C, 57·8; H, 6·0%). Light absorption in ethanol: Max. at 2210 (ε = 25,600), 2810 (ε = 19,800), inflection at 3100 Å (ε = 9300). It was undepressed in m. p. when mixed with a specimen of the authentic semicarbazone, m. p. 217—219° (decomp.) [(Schöpf *et al.*, *loc. cit.*, give m. p. 217—219° (decomp.) for the monohydrate (from ethanol)], which had absorption max., in ethanol, at 2200 (ε = 25,000), 2800 (ε = 19,000), inflection at 3100 Å (ε = 9000). The crude 6 : 7-dimethoxyindan-1-one (0·5 g.) gave with Brady's reagent the 2 : 4-*dinitrophenylhydrazone* (0·71 g.) which separated from chloroform–light petroleum (b. p. 60—80°) as bright red needles, m. p. 246—248° (decomp.) (Found: C, 55·3; H, 4·2. $C_{17}H_{16}O_6N_4$ requires C, 54·9; H, 4·3%). Light absorption in chloroform: Max. at 2490 (ε = 13,900) and 3900 Å (ε = 29,100). The compound gave no m. p. depression on mixture with a specimen [m. p. 246—248° (decomp.)] prepared from 6 : 7-dimethoxyindan-1-one prepared as described by Schöpf *et al.* (*loc. cit.*), which separated as bright red needles from chloroform–light petroleum (b. p. 60—80°) (Found: C, 55·1; H, 4·5%) and showed light absorption max., in chloroform, at 2470 (ε = 14,600) and 3910 Å (ε = 29,000).

Indan-1-*one*.—(*a*) 1 : 2-Dihydro-1-keto-2-thianaphthalene (1·4 g.) was heated under reflux for 5 hours with a suspension of Raney nickel (14 g.) in ethanol (100 c.c.). The filtered solution and ethanol washings were evaporated under reduced pressure, water (50 c.c.) was added to the residue, and the mixture steam-distilled until the distillate was free from oil. The oil solidified when the distillate was cooled in ice. The solid A (0·11 g.), m. p. 36—38°, was separated and the filtrate extracted with ether (3 × 25 c.c.), the combined extracts being dried (Na_2SO_4) and evaporated to give a further 0·42 g. of indan-1-one. Sublimation of the solid A at 60°/10^{-3} mm. gave a sublimate, m. p. 38—40° alone or mixed with an authentic specimen (m. p. 39—41°) (*Org. Synth.*, Coll. Vol. II, 336). The crude indan-1-one (0·20 g.) was converted into the semicarbazone (0·27 g.) which separated from ethanol as small prisms, m. p. 237° (decomp.) (Found: C, 63·8; H, 5·4. Calc. for $C_{10}H_{11}ON_3$: C, 63·5; H, 5·8%). Light absorption in ethanol: Max. at 2080 (ε = 17,000), 2690 (ε = 16,300), 2780 (ε = 16,200), 2990 (ε = 14,600), and 3090 (ε = 12,900), inflection at 2200 Å (ε = 12,000). The semicarbazone was not depressed in m. p. on mixture with an authentic specimen, m. p. 237° (decomp.), of indan-1-one semicarbazone [Revis and Kipping, *J.*, 1897, **71**, 238, give m. p. 239° (decomp.); von Auwers and Auffenberg, *Ber.*, 1919, **52**, 92, give m. p. 233° (decomp.)], which gave absorption max., in ethanol, at 2070 (ε = 16,100), 2700 (ε = 16,500), 2800 (ε = 16,600), 2970 (ε = 14,200), and 3080 (ε = 12,900), inflection at 2200 Å (ε = 12,700). A specimen of the crude indan-1-one (0·20 g.) with Brady's reagent gave indan-1-one 2 : 4-dinitrophenylhydrazone (0·41 g.) which separated from ethyl acetate as red rods, m. p. 258—260° (decomp.) (Found: C, 58·0; H, 3·6. Calc. for $C_{15}H_{12}O_4N_4$: C, 57·8; H, 3·85%). Light absorption in chloroform: Max. at 2500 (ε = 13,500) and 3900 Å (ε = 31,000). The derivative showed no m. p. depression when mixed with authentic indan-1-one 2 : 4-dinitrophenylhydrazone, m. p. 258—260° (decomp.) (Allen, *J. Amer. Chem. Soc.*, 1930, **52**, 2955, gives m. p. 258°; Seka and Kellermann, *Ber.*, 1942,

75, 1730, give m. p. 265°) [absorption max., in chloroform, at 2500 (ε = 15,000) and 3890 Å (ε = 30,800)].

(*b*) 1 : 2-Dihydro-1-keto-2-thianaphthalene-3-carboxylic acid (3·0 g.) was treated as above, to give crude indan-1-one (1·2 g.). This was not purified but was converted into the semicarbazone (0·61 g. from 0·5 g.) and the 2 : 4-dinitrophenylhydrazone (0·87 g. from 0·5 g.). The semicarbazone separated from ethanol as small prisms, m. p. 237° (decomp.) alone or mixed with authentic indan-1-one semicarbazone (Found : C, 63·4; H, 5·8%). Light absorption in ethanol : very similar to that recorded above. The 2 : 4-dinitrophenylhydrazone formed small red needles (from ethyl acetate), m. p. 258—260° (decomp.) undepressed on mixture with indan-1-one 2 : 4-dinitrophenylhydrazone (Found : C, 58·3; H, 3·7%). Light absorption in chloroform : Max. at 2500 (ε = 14,400) and 3880 Å (ε = 31,900).

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6. iso*Taxiresinol* * (3′-*Demethyl*iso*lariciresinol*), *a New Lignan extracted from the Heartwood of the English Yew*, Taxus baccata.

By F. E. KING, L. JURD, and T. J. KING.

A hitherto unknown lignan containing three phenolic hydroxyl groups and one methoxyl group has been isolated from the heartwood of the English yew, *Taxus baccata*. In view of the identity of the methylated lignan with *iso*lariciresinol dimethyl ether (Haworth and Kelly, *J.*, 1937, 384) it has been named *iso*taxiresinol. Oxidation of the triethyl ether to 2-(3 : 4-diethoxybenzoyl)-4-ethoxy-5-methoxybenzoic acid, which has been identified by syntheses, establishes the constitution of the new compound as 3′-demethyl*iso*lariciresinol.

From the same source a *meso*inositol monomethyl ether has been obtained which has the properties of sequoyitol found in the California redwood, *Sequoia sempervirens*.

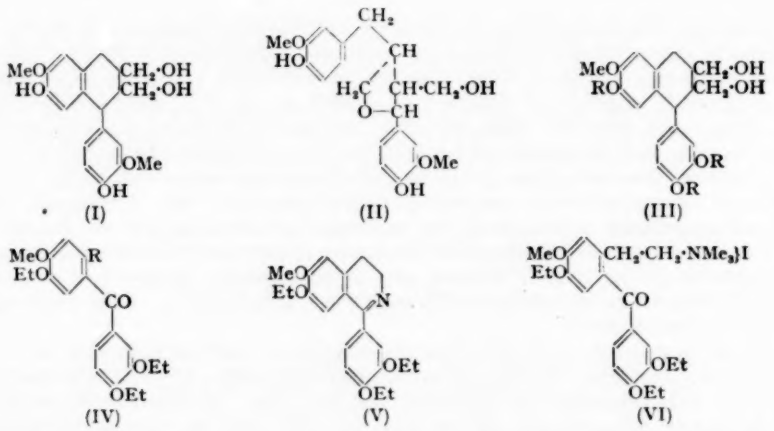
THE English yew (*Taxus baccata*) is chiefly of interest for the existence in its leaves, shoots, and berries of the incompletely formulated toxic alkaloid taxine (see Henry, "The Plant Alkaloids," 4th edn., 1949, p. 769). Associated with it is taxicatin, 3 : 5-dimethoxyphenyl-D-glucoside (Lefebvre, *J. Pharm. Chim.*, 1907, iv, **26**, 241; Merz and Preuss, *Arch. Pharm. Chim.*, 1941, **279**, 134; 1943, **281**, 205), but little is known of the products of other parts of the tree, and the timber, which is classed as a softwood and stated to be highly resistant to decay, does not appear to have been hitherto subjected to chemical investigation. The felling of a large yew tree in the grounds of the University Park, Nottingham, recently afforded an opportunity of examining the heartwood constituents, and this has led to the isolation of a new phenolic lignan which for reasons evident in the ensuing discussion has been designated *iso*taxiresinol. Present also in the aqueous extract is a monomethyl *meso*inositol believed to be identical with sequoyitol, a constituent of the California redwood, *Sequoia sempervirens*.

The lignan, amounting to 1% of the undried heartwood, together with other non-crystalline material, was removed from the powdered timber with boiling water; it was then isolated by extraction of the concentrated aqueous solution with ether, the ether-soluble portion being recrystallised from ethyl acetate and finally acetic acid until the colourless *iso*taxiresinol, $C_{19}H_{22}O_6$, m. p. 171°, was obtained. The resinol contains one methoxyl group, and the formation of crystalline alkali-insoluble trimethyl and triethyl ethers on treatment with alkyl sulphate or iodide in presence of alkali indicated the existence of three phenolic hydroxyl groups. Although acyl derivatives of *iso*taxiresinol and of its trimethyl ether could not be crystallised, the alcoholic function of the two remaining oxygen atoms was apparent from the formation of a crystalline diacetate of the *O*-triethyl*iso*taxiresinol.

* On account of the close relationship of the new lignan to *iso*lariciresinol the name "taxiresinol" is reserved in anticipation of the possible existence of an isomer similarly related to lariciresinol.—F. E. K.

When treated with hot nitric acid, the trimethyl and triethyl ethers respectively gave 4 : 5-dinitroveratrole and 4 : 5-dinitrocatechol diethyl ether, which taking into account the molecular formula and the existence of a methoxyl group demonstrated the occurrence of both a catechol and a methoxyphenol nucleus in the molecule. Oxidation of the trimethyl ether with alkaline permanganate or potassium dichromate in acetic acid gave veratroylveratric acid. From all these data it was apparent that the new natural product was a member of the lignan group. Further consideration indicated its close connexion with *iso*lariciresinol (I) which had been obtained by Haworth and Kelly (*J.*, 1937, 384) by isomerisation of lariciresinol (II), a constituent of the resinous exudate of the larch, *Larix decidua*. The properties of the trimethyl ether of the new lignan corresponded with those of *iso*lariciresinol dimethyl ether, and a direct comparison of the two ethers and of their respective anhydro-compounds proved them to be identical. We are indebted to Professor R. D. Haworth, F.R.S., for specimens of the *iso*lariciresinol derivatives.

The new lignan having been thus shown to be a demethyl*iso*lariciresinol it merely remained to determine the relative orientation of the methoxyl and the phenolic substituents. Four alternative structures are possible, *i.e.*, with the methoxyl group occupying position 6, 7, 3′, or 4′ respectively, but two of these (methoxyl at 7 or 4′) were tentatively excluded on the grounds that no known natural product in the lignan series contains an *iso*vanillyl nucleus. Furthermore, the formation of 1 : 2-diethoxy-4 : 5-dinitrobenzene as the principal nitric acid oxidation product of *iso*taxiresinol triethyl ether is more likely with the orientation of substituents shown in (III; R = Et) than if the two ethoxy groups were attached to the tetrahydronaphthalene nucleus. These considerations, though admittedly speculative, lead to (III; R = H) as the most probable structure for *iso*taxiresinol, an expression which was subsequently deduced from the nature of the triethoxymethoxybenzophenone-2-carboxylic acid, m. p. 173·5°, resulting on oxidation of the *iso*taxiresinol triethyl ether with permanganate. The constitution of this oxidation product as (IV; R = CO_2H) was duly established by syntheses, thereby affording unequivocal confirmation of (III; R = H) as the structure of *iso*taxiresinol.



Early attempts to synthesise (IV; R = CO_2H) depended on the oxidation of 5 : 3′ : 4′-triethoxy-4-methoxy-2-methylbenzophenone (IV; R = Me) prepared by the condensation of 4-ethoxy-3-methoxytoluene with 3 : 4-diethoxybenzoyl chloride, but the product was unaffected by treatment with potassium permanganate under a variety of conditions. By chromium trioxide oxidation in acetic acid solution, alone or with sulphuric acid, 3 : 6 : 7-triethoxy-2-methoxyanthraquinone and a small amount of an unidentified acid were obtained. Oxidation with potassium dichromate in boiling 75% acetic acid gave a small quantity of the anthraquinone, an alkali-insoluble substance, m. p. 152°, and an acid which after

several crystallisations had m. p. 172—173° and formed a methyl ester, m. p. 110°, not depressed on admixture with the acid and ester, respectively, obtained from *iso*taxiresinol triethyl ether. Their identity thus appeared certain, but in view of the small yield and difficult purification of the oxidation product, an alternative synthesis was devised from the more readily oxidisable 5 : 3' : 4'-triethoxy-4-methoxy-2-vinylbenzophenone (IV; R = $CH{:}CH_2$).

The necessary vinyl compound was formed by exhaustive methylation of a suitable 1-aryldihydro*iso*quinoline, for the preparation of which 2-(4-ethoxy-3-methoxyphenyl)ethylamine was condensed with 3 : 4-diethoxybenzoyl chloride giving 3 : 4-diethoxy-*N*-[2-(4-ethoxy-3-methoxyphenyl)ethyl]benzamide. 1-(3 : 4-Diethoxyphenyl)-7-ethoxy-3 : 4-dihydro-6-methoxy*iso*quinoline (V) was then obtained by ring closure of the amide with phosphoryl chloride in boiling toluene. On treating the dihydro*iso*quinoline methiodide with cold aqueous alkali and methyl iodide it was converted almost quantitatively into 2-[2-(3 : 4-diethoxybenzoyl)-4-ethoxy-5-methoxyphenyl]ethyltrimethylammonium iodide (VI), a transformation doubtless involving the usual rearrangement of a methohydroxide to a hydroxy-amine followed by ring opening to the isomeric keto-secondary base which is thereupon methylated to the quaternary salt. The various stages have been set out by Gensler and Samour (*J. Amer. Chem. Soc.*, 1950, **72**, 3318) in a summary of a parallel investigation on the synthesis of a picropodophyllin oxidation product analogous to (IV; R = CO_2H) (see also Reeve and Eareckson, *ibid.*, p. 5195), but the intermediate methiodide corresponding to (VI) was not isolated, the action of aqueous-alcoholic alkali leading directly to a vinylbenzophenone. Degradation of the methiodide (VI) was accomplished by heating it with aqueous alkali, the resulting vinylbenzophenone (IV; R = $CH{:}CH_2$) being oxidised by potassium permanganate in boiling acetone to the acid (IV; R = CO_2H). Both the acid and its methyl ester were indistinguishable from the corresponding products derived from the natural triethyl ether (III; R = Et), thus confirming the structure (III; R = H) attributed on general grounds to *iso*taxiresinol.

*iso*Lariciresinol (I) to which the new lignan is so closely analogous, appears to be known solely as a transformation product of the naturally occurring lariciresinol (II) (Haworth and Kelly, *loc. cit.*). Owing to the nature of the process used for extraction of the yew wood, *viz.*, prolonged treatment with boiling water, the possibility could not be entirely excluded that *iso*taxiresinol might also arise from a labile precursor corresponding in structure to lariciresinol. However, no evidence for the existence of an isomer of *iso*taxiresinol has been obtained; extraction of the timber with cold ether yielded, after crystallisation from ethyl acetate, only *iso*taxiresinol, though in smaller quantity than aqueous extraction, doubtless owing to the lower efficiency of the organic solvent.

After removal of *iso*lariciresinol from aqueous extracts of the wood, a highly crystalline optically inactive compound, $C_7H_{14}O_6$, m. p. 237° (yield, 0·04%) was isolated. It formed a penta-acetate, m. p. 200°, and contained one methoxyl group, and when hydrolysed gave *meso*inositol. These properties agree with those of sequoyitol, an inositol methyl ether present in the California redwood (*Sequoia sempervirens*) (Sherrard and Kurth, *J. Amer. Chem. Soc.*, 1929, **51**, 3139) and also in the Western Australian plant *Macrozamia Riedlei* (Riggs, *J.*, 1949, 3199).

Both lignans and inositols are characteristic heartwood constituents of the conifers, and the isolation of *iso*taxiresinol and of sequoyitol from the yew is of particular significance in view of the relation of the *Taxaceæ* to the coniferous trees. Flavone constituents are also widely distributed among the conifers, but compounds of this nature do not appear to occur in the heartwood of the yew. Crude ether and acetone extracts, dissolved in alcoholic hydrochloric acid, gave no characteristic colour with magnesium or zinc, thus indicating the absence of flavanols, flavanones, and flavanolones (3-hydroxy-flavanones) (Pew, *J. Amer. Chem. Soc.*, 1948, **70**, 3031).

Experimental

iso*Taxiresinol* (III; R = H).—(*a*) The heartwood used in this investigation was taken from a fully grown tree of maximum girth nearly 8 feet. Fine shavings of the timber (1000 g.) were covered with water which was boiled for 2 hours, and 3 successive extractions were thus

made, each extract being decanted and concentrated to approx. 300 c.c. After cooling and separation from the dark brown resinous precipitate (A), the aqueous solution was extracted with ether (7 × 150 c.c.). The combined ethereal solutions were dried ($MgSO_4$) and evaporated, leaving a yellow solid. This was refluxed with ethyl acetate (15 c.c.) for 10 minutes during which the mass thickened with the formation of crystalline solid. After cooling overnight, the mixture was filtered and the solid washed thoroughly with cold ethyl acetate. Most of the colour was thereby removed, the product being obtained as faintly pink, very small rods (7·7 g.), m. p. 166—169°. It was once again refluxed with ethyl acetate (15 c.c.) and, when collected (yield, 5·8 g.; m. p. 170°) and recrystallised from 2N-aqueous acetic acid, *iso*taxiresinol was obtained as almost colourless, flat rods (5·4 g.), m. p. 171° (Found: C, 65·9; H, 6·7; OMe, 9·0; active H, 1·33. $C_{19}H_{22}O_9$ requires C, 65·8; H, 6·4; OMe, 9·0; 5 active H, 1·4%). A further quantity of *iso*taxiresinol was obtained from the resinous by-product (A) which partly crystallised after several days. On addition of 40% aqueous acetic acid, the crystalline *iso*taxiresinol remained undissolved, and was collected and recrystallised from 2N-aqueous acetic acid (yield, 3 g.; m. p. 170°). *iso*Taxiresinol dissolved readily in methanol or ethanol, its solutions giving a green ferric reaction. It was moderately soluble in hot water, ether, chloroform and ethyl acetate, and sparingly soluble in benzene.

(*b*) The comminuted heartwood (200 g.) was covered with ether and left for 2 days at room temperature. The resulting solution was decanted, and the oil left on evaporation was refluxed with light petroleum. The petroleum-insoluble residue was dissolved in a small volume of hot ethyl acetate, which was filtered and set aside. A crystalline solid slowly separated and was collected after several weeks. The product (0·4 g.) had m. p. 169°, undepressed on admixture with an authentic sample of *iso*taxiresinol.

iso*Taxiresinol Trimethyl Ether* (iso*Lariciresinol Dimethyl Ether*) (III; R = Me).—Methyl sulphate (2 c.c.) was added in 2 portions during 10 minutes to a solution of *iso*taxiresinol (0·68 g.) in 2N-aqueous sodium hydroxide (12 c.c.) kept at 60°. After being heated on a steam-bath for 10 minutes the white solid (0·7 g.) was collected, dried, and recrystallised from ethyl acetate. *iso*Taxiresinol trimethyl ether separated as very small needles, m. p. 167—168°, undepressed on admixture with *iso*lariciresinol dimethyl ether. *iso*Taxiresinol trimethyl ether was also prepared by methylating taxiresinol in acetone solution with methyl iodide and potassium carbonate (yield 80%) (Found: C, 68·4; H, 7·7; OMe, 32·8. Calc. for $C_{22}H_{28}O_9$: C, 68·0; H, 7·3; 4OMe, 32·0%), $[\alpha]_D^{18}$ +19° in chloroform. Haworth and Kelly (*loc. cit.*) record for *iso*lariciresinol dimethyl ether $[\alpha]_D^{14}$ +20° in chloroform.

iso*Taxiresinol Triethyl Ether* (III; R = Et).—A solution of *iso*taxiresinol (1 g.) in ethyl iodide (5 c.c.) and acetone (20 c.c.) was refluxed with anhydrous potassium carbonate (2 g.) for 30 hours. The product solidified on removal of the solvent and addition of water, and was collected and recrystallised successively from ethanol and ethyl acetate. iso*Taxiresinol triethyl ether* (0·75 g.) separated in needles, m. p. 140° (Found: C, 69·6; H, 8·0. $C_{25}H_{34}O_6$ requires C, 69·7; H, 8·0%).

*Anhydroiso**taxiresinol Trimethyl Ether.*—*iso*Taxiresinol trimethyl ether (0·2 g.) was heated with potassium hydrogen sulphate (0·8 g.) at 180—190° for ½ hour. The solid which separated on addition of water was collected and recrystallised from methanol, anhydro*iso*taxiresinol trimethyl ether separating as colourless needles, m. p. 149·5°, alone or mixed with anhydro-*iso*lariciresinol dimethyl ether (Found: C, 71·7; H, 7·1. Calc. for $C_{22}H_{26}O_5$: C, 71·4; H, 7·1%).

*Anhydroiso**taxiresinol Triethyl Ether.*—*Anhydroiso**taxiresinol triethyl ether*, prepared in the same manner as the anhydro-trimethyl ether, separated from aqueous methanol in needles, m. p. 122·5—123° (Found: C, 72·3; H, 7·8. $C_{25}H_{32}O_5$ requires C, 72·8; H, 7·8%).

iso*Taxiresinol Triethyl Ether Diacetate.*—*iso*Taxiresinol triethyl ether (0·1 g.), anhydrous pyridine (2 c.c.), and acetic anhydride (1 c.c.) were mixed and kept at room temperature for 15 hours. After the mixture had been poured on crushed ice and set aside, the solid was collected and recrystallised from aqueous methanol. iso*Taxiresinol triethyl ether diacetate* separated in very small needles, m. p. 89·5° (Found: C, 68·1; H, 7·3. $C_{29}H_{36}O_8$ requires C, 67·8; H, 7·4).

iso*Taxiresinol Triethyl Ether Dibenzoate.*—*iso*Taxiresinol triethyl ether (0·07 g.), benzoyl chloride (0·3 c.c.), and pyridine (0·5 c.c.) were mixed and left overnight at room temperature. Water was added, and the oily solid separated by decantation was suspended in dilute aqueous ammonia for 10 minutes. When collected and washed with water, dilute hydrochloric acid and again with water, the product was dissolved in boiling alcohol. From the concentrated alcoholic solution crystals of the *dibenzoate* separated which when recrystallised from methanol

formed needles, m. p. (after drying at 100°) 125° (Found : C, 73·0; H, 6·2. $C_{39}H_{42}O_8$ requires C, 73·3; H, 6·6%).

Oxidation of iso*Taxiresinol Triethyl Ether.*—(a) *With concentrated nitric acid.* *iso*Taxiresinol triethyl ether (0·3 g.) and nitric acid (6 c.c.; *d* 1·42) were heated on a steam-bath for ½ hour; afterwards the solution was concentrated to 1 c.c., diluted with water (15 c.c.), and extracted with ether (3 × 20 c.c.). The ethereal solution was washed repeatedly with 2N-sodium carbonate until the alkaline extracts were colourless; it was then washed with water and dried. Evaporation of the ethereal solution gave a crystalline residue which was extracted several times with boiling light petroleum. The solid deposited from the petroleum extracts was recrystallised from alcohol, and separated as pale yellow plates, m. p. 110°, alone or mixed with 1 : 2-diethoxy-4 : 5-dinitrobenzene. Similarly *iso*taxiresinol trimethyl ether (0·2 g.) gave 4 : 5-dinitroveratrole (0·04 g.), m. p. and mixed m. p. 130°.

(b) *With potassium permanganate.* Powdered potassium permanganate (1·5 g.) was added during 2 hours to a refluxing solution of *iso*taxiresinol triethyl ether (0·35 g.) in acetone (40 c.c.). The solution was then concentrated to about 10 c.c., water (10 c.c.) was added, and refluxing continued until reduction of the permanganate was complete (10 minutes). Excess of water was then added, the manganese dioxide was removed by filtration, and the remaining acetone distilled from the straw-coloured filtrate, which when cooled was extracted with ether (3 × 20 c.c.). The ethereal extract was washed with 2N-sodium carbonate (2·0 c.c.) which was then added to the aqueous solution. Acidification of the latter with hydrochloric acid gave a cream-coloured precipitate which when recrystallised from aqueous methanol formed prisms, m. p. 173°, subsequently identified as 2-(*3 : 4-diethoxybenzoyl*)-*4-ethoxy-5-methoxybenzoic acid* (IV; R = CO_2H) and obtained colourless (m. p. 173·5°) when further crystallised from benzene–light petroleum (Found : C, 64·5; H, 6·0. $C_{21}H_{24}O_7$ requires C, 64·9; H, 6·2%). An excess of ethereal diazomethane and a solution of the acid in methanol gave the *methyl* ester (IV; R = CO_2Me) which separated from light petroleum in irregular plates, m. p. 111° (Found : C, 65·5; H, 6·3. $C_{22}H_{26}O_7$ requires C, 65·6; H, 6·5%). The acid gave a deep red colour with concentrated sulphuric acid and, when the solution was heated on a steam-bath, 3 : 6 : 7-triethoxy-2-methoxyanthraquinone separated as long yellow needles. The oxidation of *iso*taxiresinol trimethyl ether (0·22 g.) under similar conditions gave veratroylveratric acid (0·04 g.), m. p. 221° (lit., 221—222°), and its methyl ester, m. p. 159° (lit., 161°).

(c) *With potassium dichromate.* A mixture of *iso*taxiresinol triethyl ether (0·55 g.), potassium dichromate (2·5 g.), and glacial acetic acid (15 c.c.) was heated under reflux for 3 hours. After addition of water (100 c.c.) the mixture was extracted with ether (3 × 60 c.c.), and the washed ethereal solution extracted with 2N-sodium hydroxide. A yellow solid was precipitated on acidification of the alkaline extract and this was collected and recrystallised successively from aqueous ethanol and benzene, thus affording the acid (IV; R = CO_2H) in colourless prisms, m. p. 173° (Found : C, 64·9; H, 6·3%). Similarly, from the trimethyl ether veratroylveratric acid, m. p. 220—221°, was obtained.

3 : 4-Diethoxybenzaldehyde.—Ethyl sulphate (38·5 g.) and a solution of potassium hydroxide (18·4 g.) in water (50 c.c.) were added slowly and with stirring during 10 minutes to 3 : 4-dihydroxybenzaldehyde (13·8 g.), the reaction mixture being heated on a steam-bath. After being heated and stirred for a further 10 minutes the mixture was cooled and the oily layer extracted with ether. The washed and dried ethereal solution on distillation gave 3 : 4-diethoxybenzaldehyde as a pale yellow oil (15·4 g., 79%), b. p. 280—282° (lit., 278—280°). *3 : 4-Diethoxybenzaldehyde semicarbazone* separated from alcohol in faintly yellow needles, m. p. 175° (Found : C, 57·2; H, 6·9. $C_{12}H_{17}O_3N_3$ requires C, 57·3; H, 6·8%).

3 : 4-Diethoxybenzoic Acid.—3 : 4-Diethoxybenzaldehyde (33 g.) was added to a solution of potassium permanganate (43 g.) in water (800 c.c.), and the mixture boiled for 5 minutes to complete the oxidation. Acidification of the mixture with a stream of sulphur dioxide precipitated 3 : 4-diethoxybenzoic acid in needles, m. p. 165° (31·5 g., 88%), raised by recrystallisation from benzene–light petroleum or alcohol–water to 166—167° (lit., 165—166°) (Found : C, 62·5; H, 6·7. Calc. for $C_{11}H_{14}O_4$: C, 62·8; H, 6·7%). *3 : 4-Diethoxybenzamide* was prepared by treating the acid successively with thionyl chloride and concentrated aqueous ammonia; it separated from methanol in needles, m. p. 183·5° (Found : C, 63·5; H, 7·1; N, 6·4. $C_{11}H_{15}O_3N$ requires C, 63·1; H, 7·2; N, 6·7%).

5 : 3' : 4'-Triethoxy-4-methoxy-2-methylbenzophenone (IV; R = Me).—*O*-Ethylvanillin (25 g.) and amalgamated zinc (130 g.) were refluxed with concentrated hydrochloric acid (260 c.c.) for 20 hours; excess of zinc was then removed and the mixture steam-distilled. 4-Ethoxy-3-methoxytoluene, isolated from the distillate with ether, was thus obtained as a colourless

oil (9·3 g., 40%), b. p. 227—228° (lit., b. p. 223—224°). 3 : 4-Diethoxybenzoyl chloride was prepared from the acid and thionyl chloride, and separated from light petroleum in colourless needles.

Powdered aluminium chloride (11·1 g.) was added during 10 minutes to a solution of 4-ethoxy-3-methoxytoluene (5·45 g.) and 3 : 4-diethoxybenzoyl chloride (7·5 g.) in nitrobenzene (30 g.), the mixture being cooled in an ice-bath. After being left overnight at room temperature the solution was poured into a mixture of concentrated hydrochloric acid (10 c.c.) and crushed ice. The nitrobenzene was removed by steam-distillation and the residual oil isolated with chloroform. After washing of the extract with dilute sodium hydroxide and water, the solvent was evaporated and the oily residue dissolved in a small volume of boiling methanol. 5 : 3′ : 4′-*Triethoxy*-4-*methoxy*-3-*methylbenzophenone* (4·7 g., 40%) separated on cooling and when crystallised from methanol formed colourless prisms, m. p. 115° (Found: C, 70·2; H, 7·0. $C_{21}H_{26}O_5$ requires C, 70·4; H, 7·3%).

2-(4-*Ethoxy*-3-*methoxyphenyl*)*ethylamine*.—Ethyl sulphate (49 c.c.), and a solution of potassium hydroxide (27·6 g.) in water (60 c.c.), were added with vigorous agitation during 10 minutes to vanillin (45·6 g.) melted on a steam-bath. After a further 10 minutes' heating, the mixture was cooled and placed in a refrigerator for 3 hours. The light brown crystalline mass of *O*-ethylvanillin was ground in a mortar with a small volume of water, collected, and air-dried at 40°; the yield was 49·5 g. (90%) and the m. p. 61° (lit., 64°).

Ethylvanillin (25 g.) and malonic acid (35 g.), dissolved in pyridine (50 c.c.) and piperidine (2·5 c.c.), were heated on a steam-bath for 1 hour. After refluxing for a further 15 minutes the mixture was diluted with water, acidified with hydrochloric acid, and cooled. The precipitated 4-ethoxy-3-methoxycinnamic acid, m. p. 203° (lit., 205°), was collected and dissolved in a small volume of N-sodium hydroxide. Sodium amalgam (700 g.; 3%) was gradually added during 2½ hours with mechanical stirring and occasional additions of concentrated hydrochloric acid. The solution was decanted, treated with charcoal, filtered, and acidified, thus liberating β-(4-ethoxy-3-methoxyphenyl)propionic acid (28 g.), m. p. 130° (lit., 130°). This acid was dissolved in methanolic hydrogen chloride (150 c.c.) and refluxed for 15 hours; after removal of the solvent *in vacuo*, sodium hydrogen carbonate solution was added and the crystalline residue (27 g.) dissolved in boiling light petroleum. *Methyl* β-(4-*ethoxy*-3-*methoxyphenyl*)*propionate* separated in prisms, m. p. 37—38° (Found: C, 65·3; H, 8·0. $C_{13}H_{18}O_4$ requires C, 65·5; H, 7·6%).

The methyl ester (26 g.) was added during 5 minutes to hot 90% aqueous hydrazine hydrate. Heating was continued for 3 hours on a steam-bath and the crystalline hydrazide precipitated with water (50 c.c.). The *hydrazide* separated from benzene in prisms, m. p. 123° (Found: C, 60·2; H, 7·5; N, 11·9. $C_{12}H_{18}O_3N_2$ requires C, 60·5; H, 7·6; N, 11·8%). A solution of the hydrazide in 10% hydrochloric acid (150 c.c.) was covered with ether and cooled in ice, and sodium nitrite added slowly until present in excess. The dried ($CaCl_2$) ethereal solution was added to ethanol (50 c.c.), and the ether expelled by warming, the solution then being refluxed for 2 hours. A solution of sodium hydroxide (8·0 g.) in water (20 c.c.) was added, and after a further 15 hours' refluxing, most of the alcohol was evaporated. Water was then added and the base extracted with ether, distillation of the extract giving 2-(4-ethoxy-3-methoxyphenyl)ethylamine (5·7 g.) as an oil, b. p. 172—174°/15 mm. (lit., 165°/15 mm.). The picrate, prepared from sodium picrate and a solution of the amine in 20% aqueous lactic acid, separated from alcohol in orange-red prisms, m. p. 183° (lit., 181—182°) (Found: C, 48·3; H, 4·7; N, 13·1. Calc. for $C_{17}H_{20}O_9N_4$: C, 48·1; H, 4·7; N, 13·2).

3 : 4-*Diethoxy*-N-[2-(4-*ethoxy*-3-*methoxyphenyl*)*ethyl*]*benzamide*.—3 : 4-Diethoxybenzoyl chloride (5·2 g.) dissolved in benzene (25 c.c.) was gradually added to a solution of 2-(4-ethoxy-3-methoxyphenyl)ethylamine (4 g.) in pyridine (10 c.c.). After 15 minutes' heating on a steam-bath, most of the benzene was distilled, excess of chloroform was added, and the solution washed with 10% hydrochloric acid and with water. The chloroform was then evaporated, and the crystalline residue recrystallised from methanol. The *amide* (6·85 g., 86%) separated in needles, m. p. 148·5° (Found: C, 68·5; H, 7·7; N, 3·7. $C_{22}H_{29}O_5N$ requires C, 68·2; H, 7·5; N, 3·6%).

1-(3′ : 4′-*Diethoxyphenyl*)-7-*ethoxy*-3 : 4-*dihydro*-6-*methoxy*isoquinoline (V).—A solution of the above amide (7·5 g.) in toluene (30 c.c.) was refluxed with phosphorus oxychloride (15 g.) for 2 hours. Toluene and excess of phosphorus oxychloride were removed *in vacuo*, and the residual gum was dissolved in boiling 10% hydrochloric acid (70 c.c.). The amine hydrochloride which separated on cooling crystallised from acetone in small yellow needles, m. p. 123°. A solution of the hydrochloride in chloroform (350 c.c.) was shaken with 2N-aqueous sodium carbonate

(2 × 50 c.c.), and then washed, dried ($MgSO_4$), and evaporated. The oily residue dissolved in a small volume of boiling benzene and treated with excess of light petroleum gave 1-(3 : 4-*diethoxyphenyl*)-7-*ethoxy*-3 : 4-*dihydro*-6-*methoxy*iso*quinoline* (5·9 g., 82%), m. p. 113—114°, which when recrystallised from benzene–light petroleum formed faintly brown, rectangular prisms, m. p. 115—115·5° (Found : C, 71·5; H, 7·4. $C_{22}H_{27}O_4N$ requires C, 71·5; H, 7·4%).

A solution of the dihydro*iso*quinoline (4·3 g.) in methyl iodide (20 c.c.) was refluxed for 20 minutes and, after removal of the excess of iodide, the crystalline residue was dissolved in boiling *iso*propyl alcohol (20 c.c.). The bright yellow *methiodide* (5·9 g.), m. p. 191·5°, which separated was recrystallised from water, thus being obtained as yellow needles, m. p. 192° (Found, in a specimen dried at 100° *in vacuo* : C, 53·4; H, 6·1. $C_{23}H_{30}O_4NI,\frac{1}{2}H_2O$ requires C, 53·1; H, 6·0%).

2-[2-(3 : 4-*Diethoxybenzoyl*)-4-*ethoxy*-5-*methoxyphenyl*]*ethyltrimethyl Ammonium Iodide* (VI).—A solution of the dihydro*iso*quinoline methiodide (2 g.) in methyl iodide (12 c.c.) was shaken vigorously at room temperature with N-aqueous sodium hydroxide (80 c.c.) for 1½ hours. The methyl iodide layer was removed, the aqueous solution extracted 3 times with chloroform, and the methyl iodide and chloroform solutions were combined and dried ($MgSO_4$). The yellow gum which remained on removal of the solvent was dissolved in boiling benzene (15 c.c.) and, on addition of petroleum, a cream-coloured precipitate separated (2·1 g., 96%). Recrystallised from benzene–light petroleum, 2-[2-(3 : 4-*diethoxybenzoyl*)-4-*ethoxy*-5-*methoxyphenyl*]*ethyltrimethylammonium iodide* separated in colourless needles, m. p. 159° (Found : C, 53·9; H, 6·6; N, 3·1. $C_{25}H_{36}O_5NI$ requires C, 53·8; H, 6·5; N, 2·5%). The iodide was soluble in warm water, its solution giving a precipitate of silver iodide with silver nitrate in dilute nitric acid. The quaternary *picrate*, prepared by addition of aqueous sodium picrate to a solution of the iodide in 5% lactic acid, separated from benzene in yellow rectangular prisms, m. p. 171·5° (Found : C, 56·7; H, 5·6. $C_{31}H_{38}O_{12}N_4$ requires C, 56·5; H, 5·8%).

5 : 3′ : 4′-*Triethoxy*-4-*methoxy*-2-*vinylbenzophenone* (IV; R = $CH{:}CH_2$).—The iodide (VI) (1·8 g.), suspended in 2N-aqueous hydroxide (50 c.c.), was heated on a steam-bath for 15 minutes, whereupon trimethylamine was evolved and a yellow oil separated which solidified on cooling. This was collected and extracted 3 times with large volumes of light petroleum. The petroleum-insoluble residue, recrystallised from benzene–light petroleum, gave a small quantity of the unchanged iodide, m. p. 158—159°. The combined petroleum extracts were concentrated and deposited a crystalline mass of the *vinyl* compound (IV; R = $CH{:}CH_2$), which separated from light petroleum and from methanol in prisms (0·67 g., 56%), m. p. 118—118·5° (Found : C, 71·5; H, 7·0. $C_{22}H_{26}O_5$ requires C, 71·3; H, 7·1%).

2-(3 : 4-*Diethoxybenzoyl*)-4-*ethoxy*-5-*methoxybenzoic Acid* (IV; R = CO_2H).—(*a*) Finely powdered potassium permanganate (0·85 g.) was added gradually during 10 minutes to a solution of the vinyl compound (IV; R = $CH{:}CH_2$) (0·4 g.) in boiling acetone (60 c.c.). Refluxing was continued for 45 minutes, water (100 c.c.) was added, and most of the acetone evaporated. Manganese dioxide was removed by filtration, and the almost colourless filtrate concentrated to 50 c.c. and acidified with hydrochloric acid. The precipitated solid was collected and shaken with cold 2N-sodium carbonate, and the solution filtered from a small quantity of non-acidic material and acidified. The resulting product crystallised from aqueous methanol (0·25 g.) in colourless prisms, m. p. 173°, and recrystallisation from benzene–light petroleum gave 2-(3 : 4-diethoxybenzoyl)-4-ethoxy-5-methoxybenzoic acid as prisms, m. p. 173·5° undepressed by the acid obtained from the oxidation of *iso*taxiresinol triethyl ether (Found : C, 64·8; H, 6·2. Calc. for $C_{21}H_{24}O_7$: C, 64·9; H, 6·2%). The methyl ester, prepared by addition of excess of ethereal diazomethane to a solution of the acid in methanol, separated from light petroleum in irregular plates, m. p. 111—111·5° alone or mixed with the methyl ester of the acid derived from the natural product (Found : C, 65·9; H, 6·3. Calc. for $C_{22}H_{26}O_7$: C, 65·6; H, 6·5%). Sulphuric acid (5 drops of 89%) was added to the synthetic acid (25 mg.), and the deep red solution thus formed heated on a steam-bath for 5 minutes, by which time a mass of yellow needles separated. The mixture was diluted with water, and the yellow solid collected, washed well with acetone and recrystallised from acetic acid. 3 : 6 : 7-*Triethoxy*-2-*methoxyanthraquinone* separated in bright yellow needles, m. p. 225° (Found : C, 68·5; H, 6·2. $C_{21}H_{22}O_6$ requires C, 68·1; H, 6·0%).

(*b*) A solution of 5 : 3′ : 4′-triethoxy-4-methoxy-2-methylbenzophenone (1·0 g.) in acetic acid (30 c.c.) and water (7·5 c.c.) was refluxed with potassium dichromate (4 g.) for 3 hours. The solution was then poured into water (200 c.c.) and extracted with ether (2 × 100 c.c.). The ethereal and aqueous layers were filtered from a yellow insoluble solid (0·08 g.) which, when recrystallised from acetic acid, separated in bright yellow needles, m. p. 225°, undepressed

on admixture with 3 : 6 : 7-triethoxy-2-methoxyanthraquinone. The ethereal solution, after being washed with water (3 × 50 c.c.), was extracted with 2N-sodium carbonate (2 × 15 c.c.). Acidification of the alkaline solution precipitated a solid (0·2 g.), m. p. *ca.* 142°, which after further purification through 2N-sodium carbonate and crystallisation from aqueous methanol and benzene–light petroleum, separated in colourless prisms, m. p. 172—173°, undepressed on admixture with the acid (IV; R = CO_2H) (Found: C, 64·6; H, 6·2%). The acid gave a deep red colour with concentrated sulphuric acid and, when the solution was warmed, yellow needles of the triethoxymethoxyanthraquinone were formed. The methyl ester, prepared by the action of diazomethane, crystallised as irregular plates, m. p. and mixed m. p. 111°.

The sodium carbonate-washed ethereal solution, washed with water and evaporated, gave on crystallisation from methanol pale yellow needles (0·3 g.), m. p. 152°. This compound was not further examined.

Sequoyitol.—The yew heartwood (1200 g.) was extracted twice with cold light petroleum and twice with ether. It was then dried and steeped in cold water for 2 days, the solution then decanted, and the wood once again treated with cold water. The combined aqueous extracts were concentrated to about 150 c.c. and set aside for a day during which the brown tarry precipitate crystallised. Treatment of the solid with 40% aqueous acetic acid gave *iso*taxiresinol (2·5 g.), m. p. 170°, a further quantity being obtained by extracting the aqueous solution with ethyl acetate. The clear orange aqueous liquid was then concentrated *in vacuo* and the residual gum refluxed several times with large volumes of methanol. The combined filtered extracts were concentrated to 50 c.c. and left for 3 days, whereupon a crystalline solid separated which was collected and recrystallised from methanol. Sequoyitol (0·51 g.) was thus obtained as colourless rectangular prisms, m. p. 237° (Found: C, 42·9; H, 7·5; OMe, 15·7. Calc. for $C_7H_{14}O_6$: C, 43·3; H, 7·3; 1OMe, 16·0%). The compound (0·05 g.) was demethylated by refluxing it with hydriodic acid (1·0 c.c.; *d* 1·7) for 1 hour. The product separated from aqueous ethanol in colourless prisms, m. p. 221°, alone or mixed with authentic *meso*inositol. The acetate had m. p. 215° alone or mixed with *meso*inositol hexa-acetate.

Sequoyitol Penta-acetate.—The cyclitol (0·1 g.), fused sodium acetate (0·2 g.), and acetic anhydride (5 c.c.) were heated on a steam-bath for 2 hours. Excess of water was added and the mixture warmed to destroy excess of anhydride. The solvent was then removed *in vacuo*, and the addition of water gave sequoyitol penta-acetate (0·18 g.) which, when recrystallised successively from benzene–light petroleum and alcohol, separated as colourless needles, m. p. 200° (Found: C, 50·4; H, 5·7; OMe, 8·5; OAc, 49·4. Calc. for $C_{17}H_{24}O_{11}$: C, 50·5; H, 6·0; 1OMe, 7·8; 5OAc, 53·2%).

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THE UNIVERSITY, NOTTINGHAM. [*Received, August 18th*, 1951.]

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7. *Amino-acids and Peptides. Part VI.* Synthesis of* L-*Aspartyl Peptides from Carbobenzyloxy-*L-*aspartic Anhydride.*

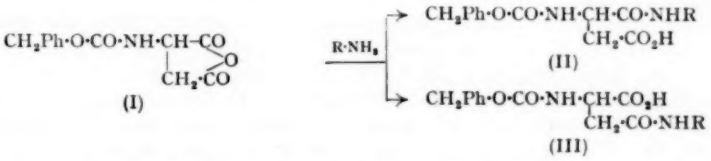
By W. J. LE QUESNE and G. T. YOUNG.

The reaction of carbobenzyloxy-L-aspartic anhydride (I) with amino-acid esters has been found to yield both α- and β-aspartyl derivatives (II and III) which may be separated by fractional extraction with alkali. In this way α- and β-L-aspartyl-glycine, -L-tyrosine, and -L-glutamic acids have been prepared. From the products of the reaction of the anhydride with ethanol, crystalline α-ethyl carbobenzyloxy-L-aspartate has been isolated. The β-aspartyl peptides are distinguishable from their α-isomers in that they give a blue colour with ninhydrin.

IN Part I (Le Quesne and Young, *J.*, 1950, 1954) it was shown that the reaction of carbobenzyloxy-L-glutamic anhydride with amino-compounds and with alcohols gives both α- and γ-glutamyl derivatives, the former normally preponderating. Carbobenzyloxy-L-aspartic anhydride (I) presents an analogous problem. There has long been evidence

* Part V, *J.*, 1951, 3047.

that this anhydride ring may open to yield both α- and β-aspartyl compounds (II and III). Although Bergmann and Zervas (*Ber.*, 1932, **65**, 1192) reported that with ammonia carbobenzyloxy-L-*iso*asparagine (II; R = H) was formed, with L-tyrosine ethyl ester



hydrochloride in pyridine solution they obtained a substance subsequently shown (Bergmann, Zervas, Salzmann, and Schleich, *Z. physiol. Chem.*, 1934, **224**, 17) to be β-L-aspartyl-L-tyrosine ethyl ester, together with a low-melting material which was not investigated further. From the reaction with glycine ethyl ester, Grassmann and Schneider (*Biochem. Z.*, 1934, **273**, 452) obtained carbobenzyloxy-α-L-aspartylglycine ethyl ester of melting point 113°; Bergmann, Zervas, and Fruton later (*J. Biol. Chem.*, 1935, **111**, 225) raised the melting point to 128° by repeated crystallisation, and it seemed likely that the contaminant was the β-isomer.

We have therefore reinvestigated the reaction of carbobenzyloxy-L-aspartic anhydride with amino-acid esters. The preparation of the anhydride itself calls for some comment; Bergmann and Zervas (*loc. cit.*) originally gave its melting point as 84°, but a year later (Bergmann, Zervas, and Salzmann, *Ber.*, 1933, **66**, 1288) this figure was corrected to 124°. Subsequently, Miller, Behrens, and du Vigneaud (*J. Biol. Chem.*, 1941, **140**, 411) used cold acetic anhydride to effect the ring closure but recorded no constants for their product. Using the latter method we obtained an anhydride melting, after recrystallisation, at 109—111°; more recently, in this laboratory, Mr. J. B. Capindale has obtained, by a slightly modified procedure (to be published), material melting sharply at 111°.

The products of the reaction of the anhydride with glycine ethyl ester in ethyl acetate solution were separated by fractional extraction with aqueous sodium carbonate (compare Part I, *loc. cit.*); β-aspartyl peptides, having the α-carboxyl group free, would be expected to be slightly stronger acids than their α-isomers. Both carbobenzyloxy-α- and -β-L-aspartylglycine ethyl ester were isolated in this manner from the reaction products, in nearly equal yield and having very similar melting points; the mixed melting point was depressed. Hydrolysis gave carbobenzyloxy-α- and -β-L-aspartylglycine (again similar in melting point) and hydrogenation yielded α- and β-L-aspartylglycine.

The latter dipeptide was prepared earlier by another route by Grassmann and Schneider (*loc. cit.*) who recorded a melting point of 153° for the anhydrous compound and $[\alpha]_D^{22}$ +7·2° for the monohydrate (in water containing one mol. of hydrochloric acid). Our product melted initially at 153—156°, but analysis showed it to be the monohydrate; drying gave the anhydrous material melting at 190—200° (with decomposition). Moreover, our hydrate had $[\alpha]_D^{21}$ +13·9° ± 0·8°, in a similar solvent. Our constants for α-L-aspartylglycine agree with those of Grassmann and Schneider.

From the products of the reaction of the anhydride with L-tyrosine ethyl ester in ethyl acetate solution we have isolated the new compound, carbobenzyloxy-α-L-aspartyl-L-tyrosine ethyl ester, together with its β-isomer. Hydrolysis gave carbobenzyloxy-α-L-aspartyl-L-tyrosine, which on hydrogenation gave α-L-aspartyl-L-tyrosine as the monohydrate. Under similar conditions diethyl L-glutamate gave the previously unreported diethyl carbobenzyloxy-α- and β-L-aspartyl-L-glutamates, and thence the isomeric carbobenzyloxy-L-aspartyl-L-glutamic acids and the corresponding dipeptides.

It would appear therefore that this method of synthesis leads normally to a mixture of α- and β-aspartyl derivatives, which may be separated by fractional extraction with alkali.

Alcohols and alkoxides would similarly be expected to yield both α- and β-esters. Pauly and Weir (*Ber.*, 1910, **43**, 661) obtained α-methyl benzoyl-L-aspartate from benzoyl-L-aspartic anhydride and methanol; and Bergmann, Zervas, and Salzmann (*loc. cit.*)

prepared crystalline α-benzyl carbobenzyloxy-L-aspartate in an analogous manner. By the action of sodium methoxide on toluene-*p*-sulphonyl-L-aspartic anhydride, Harington and Moggridge (*J.*, 1940, 707) isolated a substance provisionally assigned the structure of the α-ester, by analogy.

The product obtained by heating carbobenzyloxy-L-aspartic anhydride with ethanol was fractionally extracted with aqueous sodium carbonate. The early fractions failed to crystallise, but later extracts yielded crystalline α-ethyl carbobenzyloxy-L-aspartate, which on hydrogenation gave α-ethyl L-aspartate. With aqueous ammonia the former compound gave carbobenzyloxy-L-*iso*asparagine of melting point 167—169° (uncorrected); Bergmann and Zervas's original product, obtained by direct ring-opening of the anhydride with ammonia, had melting point 164° (corrected).

It is of interest that although the α-aspartyl peptides so far prepared by us give a normal purple colour with ninhydrin, that from the β-isomers is blue.

Experimental

M. p.s are uncorrected. Combustion analyses are by Drs. Weiler and Strauss and by Mr. F. C. Hall.

Carbobenzyloxy-L-aspartic Anhydride.—The method of Miller, Behrens, and du Vigneaud (*loc. cit.*) was used. The crude product was dried in a vacuum-desiccator over calcium chloride and sodium hydroxide and purified by extraction with ether in a Soxhlet apparatus. Recrystallisation from acetone–ether–light petroleum gave m. p. 109—111°.

Carbobenzyloxy-α- and -β-L-aspartylglycine Ethyl Esters.—Carbobenzyloxy-L-aspartic anhydride (4·0 g.) in ethyl acetate (20 ml.) was added to glycine ethyl ester (from 8·5 g. of hydrochloride) in ethyl acetate (50 ml.) slowly, with shaking. After this had been kept overnight, aqueous hydrochloric acid was added; after shaking, the ethyl acetate layer was separated, washed with water, and then extracted with successive portions of aqueous sodium carbonate (each containing 0·12 g.). On acidification, the products obtained rapidly solidified in all cases. The fractionation was repeated on the first three fractions, giving *carbobenzyloxy-β-L-aspartylglycine ethyl ester* (1·2 g., 21%), m. p. 122—124°, unchanged by recrystallisation from chloroform–ether (Found: C, 54·6; H, 5·9; N, 7·7. $C_{16}H_{20}O_7N_2$ requires C, 54·5; H, 5·7; N, 8·0%). The mixed m. p. with the α-isomer was 104—108°.

From the later fractions by a further fractionation was obtained carbobenzyloxy-α-L-aspartylglycine ethyl ester (1·1 g., 19%), m. p. 120—122° (Found: C, 54·7; H, 5·8; N, 8·0%).

Carbobenzyloxy-β-L-aspartylglycine.—Carbobenzyloxy-β-L-aspartylglycine ethyl ester (1 g.) in N-sodium hydroxide (7 ml.) was kept at 14° for 1½ hours and acidified with 5N-hydrochloric acid. The precipitated carbobenzyloxy-β-L-aspartylglycine (0·7 g., 75%) was filtered off after 1 hour at 0°; it had m. p. 158—161°, raised to 160—162° by recrystallisation from ethyl acetate.

β-L-*Aspartylglycine.*—Carbobenzyloxy-β-L-aspartylglycine (0·5 g.) in aqueous methanol was hydrogenated at atmospheric pressure in the presence of palladium black, giving β-L-aspartylglycine monohydrate (0·30 g., 93%), m. p. 153—156°, $[\alpha]_D^{20}$ +13·9° (*c*, 2·59 in water containing 1 mol. of hydrochloric acid) (Found: C, 34·7; H, 5·6; N, 14·0. Calc. for $C_6H_{10}O_5N_2,H_2O$: C, 34·6; H, 5·8; N, 13·5%). After drying for 4 hours at 110° and 18 mm. it had m. p. 190—200° (decomp.).

α-L-*Aspartylglycine.*—Carbobenzyloxy-α-L-aspartylglycine ethyl ester was hydrolysed to carbobenzyloxy-α-L-aspartylglycine, m. p. 162—165°, which was hydrogenated as above, giving α-L-aspartylglycine, m. p. 184—185°, $[\alpha]_D^{20}$ +35·0° (*c*, 2·14 in water containing 1 mol. of hydrochloric acid).

Carbobenzyloxy-α- and -β-L-aspartyl-L-tyrosine Ethyl Esters.—Carbobenzoyloxy-L-aspartic anhydride (2 g.) in ethyl acetate (10 ml.) was added portionwise, with shaking, to tyrosine ethyl ester (3·8 g.) in ethyl acetate (40 ml.). After being kept overnight, the solution was washed with hydrochloric acid and water and then extracted with successive portions of aqueous sodium carbonate (each containing 0·08 g.). The oils obtained on acidification of the earlier fractions crystallised when washed with ether, giving carbobenzyloxy-β-L-aspartyl-L-tyrosine ethyl ester (0·55 g., 15%), m. p. 191—196°, raised to 200—201° by recrystallisation from ethanol.

The later fractions, and the material extracted from the earlier ones with ether, were combined and recrystallised from ethyl acetate–light petroleum, giving *carbobenzyloxy-α-L-aspartyl-L-tyrosine ethyl ester* (1·5 g., 40%), m. p. 142—144° (Found: C, 59·9; H, 5·9; N, 6·4. $C_{23}H_{26}O_6N_2$ requires C, 60·2; H, 5·7; N, 6·1%).

Carbobenzyloxy-α-L-aspartyl-L-tyrosine.—Carbobenzyloxy-α-L-aspartyl-L-tyrosine ethyl ester (1 g.) in N-sodium hydroxide (7 ml.) was left for 2 hours at 17°. After acidification with 5N-hydrochloric acid and storage for 1 hour at 0°, the *carbobenzyloxy-α-L-aspartyl-L-tyrosine* (0·9 g., 96%) was filtered off. It was dried in a vacuum-desiccator over phosphoric oxide and recrystallised from ethyl acetate–light petroleum; it then had m. p. 149—151° (Found: C, 58·7; H, 4·8; N, 6·3. $C_{21}H_{22}O_8N_2$ requires C, 58·6; H, 5·1; N, 6·5%).

α-L-Aspartyl-L-tyrosine.—Carbobenzyloxy-α-L-aspartyl-L-tyrosine (0·7 g.) was hydrogenated in aqueous methanol in the usual fashion. Evaporation of the solution (to approx. 1 ml.) and addition of acetone (25 ml.) gave *α-L-aspartyl-L-tyrosine monohydrate* (0·35 g., 68%), m. p. 177—178°, which retained its water after an hour at 100°/18 mm. (Found: C, 49·3; H, 5·9; N, 8·8. $C_{13}H_{16}O_6N_2,H_2O$ requires C, 49·7; H, 5·8; N, 8·9%); it had $[\alpha]_D^{21}$ +17·8° (*c*, 2·59 in water containing 1 mol. of hydrochloric acid).

Diethyl Carbobenzyloxy-α- and -β-L-aspartyl-L-glutamates.—Carbobenzyloxy-L-aspartic anhydride (3 g.) in ethyl acetate (15 ml.) was added slowly, with shaking, to a solution of diethyl L-glutamate (from 9 g. of hydrochloride) in ethyl acetate (50 ml.). Next morning the solution was washed with dilute hydrochloric acid and water, then extracted with successive portions of aqueous sodium carbonate (each containing 0·12 g.). All the fractions obtained by acidification slowly crystallised; the middle ones were refractionated. Recrystallisation from ethyl acetate–light petroleum gave *diethyl carbobenzyloxy-α-L-aspartyl-L-glutamate monohydrate* (1·2 g., 21%), m. p. 82—84° (Found: C, 53·8; H, 6·4; N, 6·0. $C_{21}H_{28}O_9N_2,H_2O$ requires C, 53·6; H, 6·4; N, 6·0%), and the *β-isomer* (*monohydrate*) (1·1 g., 19%), m. p. 71—73° (Found: C, 53·7; H, 6·3; N, 6·1%); the mixed m. p. of the two isomers was 62—68°.

Carbobenzyloxy-α-L-aspartyl-L-glutamic Acid.—Diethyl carbobenzyloxy-α-L-aspartyl-L-glutamate (0·5 g.) in N-sodium hydroxide (5 ml.) was set aside for 1 hour and acidified with 5N-hydrochloric acid. After being kept at 0° the *carbobenzyloxy-α-L-aspartyl-L-glutamic acid* (0·3 g., 71%) was filtered off; it had m. p. 151—152° (Found: C, 51·9; H, 5·2; N, 7·2. $C_{17}H_{20}O_9N_2$ requires C, 51·5; H, 5·1; N, 7·1%).

α-L-Aspartyl-L-glutamic Acid.—Carbobenzyloxy-α-L-aspartyl-L-glutamic acid (0·4 g.) in aqueous methanol was hydrogenated in the normal manner. Evaporation of the filtrate under reduced pressure (to 1 ml.) and addition of ethanol (25 ml.) precipitated *α-L-aspartyl-L-glutamic acid* (0·2 g., 75%); it was recrystallised from aqueous ethanol and dried at 100° and 18 mm., then having m. p. 150—155°, $[\alpha]_D^{25}$ +5·6° (*c*, 3·39 in water) (Found: C, 40·7; H, 5·7; N, 10·7. $C_9H_{14}O_7N_2$ requires C, 41·2; H, 5·4; N, 10·7%).

Carbobenzyloxy-β-L-aspartyl-L-glutamic Acid.—Diethyl carbobenzyloxy-β-L-aspartyl-L-glutamate (0·5 g.) in N-sodium hydroxide (5 ml.) was left for 1½ hours acidified with 5N-hydrochloric acid and cooled to 0° for 15 minutes. The *carbobenzyloxy-β-L-aspartyl-L-glutamic acid* (0·3 g., 71%) was filtered off: it had m. p. 153—157° unaltered by recrystallisation from hot water (Found: C, 51·8; H, 5·2; N, 7·5. $C_{17}H_{20}O_9N_2$ requires C, 51·5; H, 5·1; N, 7·1%).

β-L-Aspartyl-L-glutamic Acid.—Carbobenzyloxy-β-L-aspartyl-L-glutamic acid (0·1 g.) in aqueous methanol was hydrogenated in the normal manner. The filtrate was evaporated to dryness and absolute ethanol added; *β-L-aspartyl-L-glutamic acid* separated as a white solid (0·02 g.). Analysis was made difficult by the extreme hygroscopicity of the product which was dried at 100°/18 mm. (Found: C, 37·8; H, 5·5; N, 9·5. $C_9H_{14}O_7N_2,H_2O$ requires C, 38·6; H, 5·7; N, 10·0%).

Paper-partition Chromatography of some Aspartyl Peptides.—By use of phenol saturated with water in an atmosphere containing ammonia, the following R_F values were obtained; each peptide gave a single spot with ninhydrin, purple in the case of the α-peptides and blue from the β-isomers: α-L-aspartyl-glycine 0·13, -L-tyrosine 0·26, and -L-glutamic acid 0·05; β-L-aspartyl-glycine 0·16, -L-tyrosine 0·29, and -L-glutamic acid, 0·05.

α-Ethyl Carbobenzyloxy-L-aspartate.—Carbobenzyloxy-L-aspartic anhydride (4·0 g.) and ethanol (25 ml.) were heated in a sealed tube at 120—130° for 4 hours. The resulting solution was evaporated under reduced pressure, dissolved in ether, and extracted with successive portions of aqueous sodium carbonate; the later fractions crystallised on acidification, to give *α-L-ethyl carbobenzylcxy-L-aspartate* which was recrystallised from ether–light petroleum, then having m. p. 80—82° (1·55 g., 33%) (Found: C, 57·0; H, 5·9; N, 4·7. $C_{14}H_{17}O_6N$ requires C, 57·0; H, 5·8; N, 4·7%). The earlier fractions gave oils which did not crystallise.

α-Ethyl L-Aspartate.—α-Ethyl carbobenzyloxy-L-aspartate (0·5 g.) in aqueous ethanol was hydrogenated in the usual manner. The filtrate was evaporated under reduced pressure (to 1 ml.) and ethanol (25 ml.) added. After ½ hour the *α-ethyl L-aspartate* (0·2 g., 74%) was filtered off; it had m. p. 180—181°, raised to 181—183° by recrystallisation from 80% aqueous

ethanol (Found: C, 44·7; H, 7·0; N, 8·4. $C_6H_{11}O_4N$ requires C, 44·7; H, 6·9; N, 8·7%); the mixed m. p. with β-ethyl L-aspartate was 151—157°.

Carbobenzyloxy-L-iso*asparagine*.—A solution of α-ethyl carbobenzyloxy-L-aspartate (0·5 g.) in aqueous ammonia (*d* 0·880; 5 ml.) was set aside for 4 days and then acidified, with cooling, by dropwise addition of concentrated hydrochloric acid. After 1 hour, the solution was filtered and the carbobenzyloxy-L-*iso*asparagine recrystallised from hot water, giving needles of m. p. 165—167° (0·3 g., 67%), raised to 167—169° by another crystallisation. The mixed m. p. with carbobenzyloxy-L-asparagine (m. p. 157—159°) was 153—157°.

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8. *Kinetics and Mechanism of Aromatic Nitration. Part IX.* Nitrosating Agents in Nitrations catalysed by Nitrous Acid.*

By EILEEN L. BLACKALL, E. D. HUGHES, and C. K. INGOLD.

The kinetics of nitration of *p*-chloroanisole by nitric acid in acetic acid under catalysis by nitrous acid (Part VI) has been further studied, using conditions which isolate the nitration process dependent on catalysis by nitrous acid, a catalysis believed to operate by preliminary nitrosation of the aromatic molecule (Part VI). The object was to determine the active nitrosating agents, and the method has been to examine the kinetic effect of added ionising nitrates and of water. The analysis shows that two nitrosating agents are at work, the nitrosonium ion and the dinitrogen tetroxide molecule, the former being specifically more than ten times faster in reaction than is the latter.

THE catalysis, by nitrous acid, of the nitration of aniline and phenol and their derivatives, and also, as was shown, of other sufficiently reactive aromatic compounds, has already been studied kinetically (Part VI, *J.*, 1950, 2628), most thoroughly in the examples of the nitration of *p*-chloroanisole and of *p*-nitrophenol by nitric acid in constant excess in solvent acetic acid. It was thus shown that the total nitration process consists of two parts, which could be isolated. One was the now familiar nitronium-ion reaction, which is anti-catalysed by nitrous acid, according to the law,

$$\text{Rate} = k_0(1 + a[HNO_2]^{1/2})^{-1} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (1)$$

and the other was a kinetically new reaction of the form,

$$\text{Rate} = k_2[ArH][HNO_2] \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (2)$$

to which the positive catalysis of nitration is due. The latter reaction could be made negligible by cutting out the nitrous acid as completely as possible, and raising the concentration of nitric acid sufficiently to render the nitronium ion freely available; or it could be made dominating by providing an ample supply of nitrous acid, and so reducing the concentration of nitric acid as to repress strongly the production of nitronium ions. It will be understood that by "nitrous acid" and by "HNO_2" is here meant stoicheiometric nitrous acid, that is, the whole of the material, which in the water-diluted solution, could be collectively estimated as nitrous acid.

The observed kinetic form of the reaction responsible for the catalysis was regarded as giving strong support to a theory, which had often been inconclusively discussed, namely, that catalysed nitration depends on initial nitrosation, followed by a rapid oxidation of the

* Part VIII, *J.*, 1950, 2678.

formed nitroso-compound, with restoration of as much nitrous acid as was consumed in the original slow stage :

$$\left.\begin{aligned} \mathrm{ArH} + \mathrm{HNO_2} &\xrightarrow{\text{slow}} \mathrm{ArNO} + \mathrm{H_2O} \\ \mathrm{ArNO} + \mathrm{HNO_3} &\xrightarrow{\text{fast}} \mathrm{ArNO_2} + \mathrm{HNO_2} \end{aligned}\right\} \quad . \quad . \quad . \quad . \quad . \quad . \quad (\mathrm{I})$$

Here again " nitrous acid " and " HNO_2 " mean stoicheiometric nitrous acid.

The obvious next step in the development of this theory was to identify the nitrosating agent; and this is the object of the present paper. Possible nitrosating agents are the nitrosonium ion, and any accessible molecule or ion of the form NOX, where X is a strong electron-attractor—any " nitrosonium-ion carrier," as we call such compounds. At the outset, the species NO^+, $H_2NO_2^+$, N_2O_4, N_2O_3, and HNO_2, to list only the more obvious, all qualify for consideration. For although the free nitrosonium ion should be the most reactive of these, so much more of one of the others may be present in the system employed, as to give that one a dominating kinetic importance. Moreover concurrent investigations of *O*- and *N*-nitrosation, diazotisation, and deamination lead to the belief that several of the compounds listed above can act as nitrosating agents under appropriate conditions (cf. Hughes, Ingold, and Ridd, *Nature*, 1950, **166**, 642; Yoffe and Gray, *J.*, 1951, 1412). It will be understood that the chemical names and formulæ in this paragraph, contrary to the preceding usage, mean what they look like, HNO_2 referring to molecular nitrous acid, N_2O_4 to undissociated dinitrogen tetroxide, and so on.

It is first necessary to consider, for each of the possible nitrosating agents, whether, and if so with what restrictions, a rate-determining nitrosation by that agent, followed by rapid oxidation of the nitroso-compound as described (reactions I), would lead to a catalysed nitration of the observed kinetic form (equation 2), in the experimental conditions of the kinetic investigation.

It was shown kinetically in Part II (*J.*, 1950, 2400), and the conclusion was confirmed by a study of physical properties, that, in the presence of a great excess of nitric acid in organic solvents such as acetic acid, stoicheiometric nitrous acid is stored mainly as molecular dinitrogen tetroxide. That being so, an assumption that the effective nitrosonium-ion carrier, in the conditions of the kinetic experiments, is dinitrogen tetroxide would clearly be consistent with the kinetics : molecular dinitrogen tetroxide passes the kinetic test unconditionally.

However, there are other, if conditional, possibilities. It was shown in Part II that, in the presence of excess of nitric acid in a solvent such as acetic acid, the dinitrogen tetroxide molecule is slightly ionised to the nitrosonium ion and the nitrate ion (cf. Goulden and Millen, *J.*, 1950, 2620) :

$$\mathrm{N_2O_4} \rightleftharpoons \mathrm{NO^+} + \mathrm{NO_3^-} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (\mathrm{II})$$

Now this nitrate ion could be, and indeed is likely to be, thus produced in amounts considerably smaller than the buffered quantity provided by the various ionisation equilibria of nitric acid, for example, the equilibrium,

$$\mathrm{HNO_3} + \mathrm{HOAc} \rightleftharpoons \mathrm{NO_3^-} + \mathrm{H_2OAc^+} \quad . \quad . \quad . \quad . \quad . \quad (\mathrm{III})$$

It follows that, provided the ionisation of dinitrogen tetroxide is not so extensive as to upset the buffering of nitrate ion, the concentration of free nitrosonium ion will bear a constant ratio to that of molecular dinitrogen tetroxide, and therefore to that of stoicheiometric nitrous acid. Therefore, given the condition stated, the hypothesis of nitrosation by the free nitrosonium ion is consistent with the observed kinetics.

We have no exact knowledge of the equilibria of self- or mutual dehydration which determine the active mass of water in these nearly anhydrous solutions. But if, by any means, this quantity were buffered sufficiently to experience no disturbance from equilibria involving molecular nitrous acid, then an assumption that nitrosation takes place by an attack of this molecule on the aromatic substance can easily be shown to be in agreement with the kinetics.

Finally, if conditions of both the stated types were simultaneously fulfilled, in particular, if the active masses of both nitrate ion and water were so buffered that neither suffered disturbance from equilibria involving the nitrous acidium ion, then the hypothesis that this ion is the nitrosating agent can be seen to accord with the kinetics.

Thus, of the listed nitrosating agents, all satisfy, or might satisfy, the already ascertained kinetic laws, with the single exception of dinitrogen trioxide, which, at best, would require the stoicheiometric nitrous acid to enter through the square of its concentration, instead of through the first power, into the rate equation.

We have sought to distinguish between the remaining possibilities by examining the kinetic effect of nitrate ion and of water, added in quantities greater than those produced from nitric acid, or from the catalysing nitrous acid. According to the identity of the nitrosating agent, the second-order rate constant k_2 for catalysed nitration should then suffer modification according to the law

$$k_2 \propto [NO_3^-]^x[H_2O]^y$$

where the powers x and y have the following values for the nitrosating agents under consideration :

	NO^+	$H_2NO_2^+$	N_2O_4	HNO_2
x	−1	−1	0	0
y	0	+1	0	+1

Clearly it should be possible to obtain the required distinction on this basis.

It has been found that the rate of the catalysed reaction, as expressed by the second-order constant k_2 of equation (2), is strongly depressed by added ionising nitrates. Added water exerts a relatively trivial retarding effect, which we shall neglect, believing it to arise from less direct causes than are of present concern. This much was indicated in Part VI, though only in experiments at higher and more scattered solute concentrations than are quantitatively significant. It has been found, moreover, that the alkali-metal nitrates formerly employed are not fully dissociated in the medium at kinetically useful concentrations. And, therefore, in developing the work we have gone over to tetra-alkylammonium nitrates, which seem to be free from this difficulty.

A quantitative study of the kinetic effect of nitrates has shown that the rate-constant k_2 of catalysed nitration is additively composed of two parts : one part is unaffected by nitrates (x zero) ; the other is diminished by added nitrates (x negative). This result, together with the relative indifference of the rate to water (y zero), suggests the hypothesis that the total nitrosating activity is being shared between two agents, namely, the dinitrogen tetroxide molecule and the nitrosonium ion.

A first kinetic expression of this hypothesis is as follows :

$$\text{Rate} = k_2'[\text{ArH}][N_2O_4] + k_2''[\text{ArH}][NO^+]$$

Since in organic solvents containing nitric acid, dinitrogen tetroxide is only slightly ionised, the above equation can be rewritten thus :

$$\text{Rate} = [\text{ArH}][N_2O_4]\{k_2' + (Kk_2'')/[NO_3^-]\}$$

K being the ionic dissociation constant of dinitrogen tetroxide. Since the concentration of molecular dinitrogen tetroxide, as it appears in these equations, must be nearly identical with the concentration of stoicheiometric nitrous acid, as contained in equation (2), our expression for the above hypothesis of dual action can be put in the following form :

$$k_2 = k_2' + (Kk_2'')/[NO_3^-] \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (3)$$

When testing equation (3), one has to deal with the slight complication that the concentration of nitrate ion which here appears is not simply the known concentration of added salt, but is the total concentration of nitrate ion present in the medium, including the not exactly known portion derived from the ionisation of nitric acid and nitrous acid. The procedure employed is best explained with reference to some actual measurements.

The table records a set of data for the effect of added tetramethylammonium nitrate and tetraethylammonium nitrate on the rate of nitration of *p*-chloroanisole by nitric acid in

fixed concentration in acetic acid at a fixed temperature, under catalysis by stoicheiometric nitrous acid. With the chosen concentrations of nitric acid and of nitrous acid, equation (1), representing nitronium-ion nitration, is entirely unimportant; and equation (2),

Effect of added tetramethylammonium and tetraethylammonium nitrates on rate of nitration of p-*chloroanisole by* 4·44M-*nitric acid in acetic acid at* 35·0° *under catalysis by nitrous acid.*

(Initially, $[Cl{\cdot}C_6H_4{\cdot}OMe] \sim 0{\cdot}08$M.; k_1 in sec.$^{-1}$; k_2 in sec.$^{-1}$ mole^{-1} l.)

Run no.	[Nitrate]	$[HNO_2]$	10^4k_1	10^3k_2
12	None	0·075	26·1	34·9
	$NMe_4^+NO_3^-$			
16	0·0070	0·058	17·3	29·9
18	0·0097	0·050	13·6	27·0
26	0·0126	0·024	6·15	25·9
17	0·0167	0·058	13·7	23·5
14	0·0208	0·062	13·3	21·4
15	0·0234	0·066	13·8	21·0
19	0·0800	0·048	7·40	15·4
	$NEt_4^+NO_3^-$			
27	0·0242	0·025	4·88	19·8
	0·0115	0·021	5·17	25·2

representing the catalysed reaction, expresses the whole of the rate. Since nitrous acid is neither formed nor destroyed, each kinetic run yields a first-order rate constant k_1, from which, by division by the concentration of nitrous acid present during the run, the second-order constant k_2 of equations (2) and (3) can be deduced.

FIG. 1.

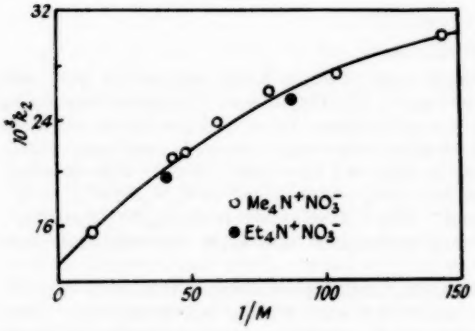
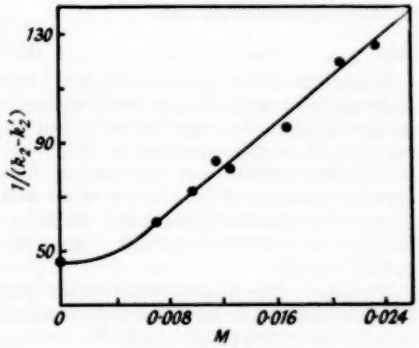


FIG. 2.



The first step in the reduction of these data is to plot k_2, the second-order rate constant, against 1/M., the reciprocal of the concentration of *added* nitrate. The resulting graph is in Fig. 1; and from it a limiting value of k_2 at $(1/\text{M.}) = 0$ can be read. Now although this graph will not strictly coincide with the graph that we cannot plot of k_2 against $1/[NO_3^-]$, where NO_3^- means *total* nitrate ion, as in equation (3), the two graphs should merge at the high-concentration end, and hence should run to the same limit at $(1/\text{M.}) = 0 = (1/[NO_3^-])$. Thus the observed limit of k_2 at $(1/\text{M.}) = 0$, namely 13×10^{-3} sec.$^{-1}$ mole^{-1} l., should be the value of k_2' in equation (3), that is, the rate constant for nitrosation by molecular dinitrogen tetroxide.

This value of k_2' is now used, in conjunction with each determined value of k_2, for the purpose of constructing a graph of $1/(k_2 - k_2')$ against M. Bearing in mind the form of equation (3), and the circumstance that M. and $[NO_3^-]$ must converge to congruence at higher concentrations, one sees that this graph should be asymptotic to a straight line, and that the reciprocal of its limiting slope should be equal to Kk_2''. The actual graph is in Fig. 2, and it has the expected asymptotically linear form. From its steady slope, the value $2{\cdot}5 \times 10^{-4}$ sec.$^{-1}$ can be deduced for Kk_2''.

This composite constant, the product of the dissociation constant of dinitrogen tetroxide and the rate constant for nitrosation by nitrosonium ion, cannot be exactly factorised on the basis of the present data. Yet the analysis can be pushed a little further as follows.

First, the value of k_2 observed in the absence of any added salt, combined with values of k_2' and of Kk_2'' given above, enable equation (3) to be used for the purpose of deducing the related value of $[NO_3^-]$, that is, the concentration of nitrate ion present in the salt-free nitration medium. This value is 0·011M. Under the extreme (but probably approximate) assumption that all this comes from nitric acid (reaction III) and none from dinitrogen tetroxide (reaction II), it would represent, as an upper limit, a degree of dissociation of only 0·2% of the 4·44M.-nitric acid in acetic acid at 35°, consistently with our previous knowledge that nitric acid behaves as a weak electrolyte in solvent acetic acid.

Secondly, under the other extreme (probably not approximate) assumption, that the whole of the nitrate ion comes from dinitrogen tetroxide (reaction II), and none of it from nitric acid (reaction III), we may derive an upper limit for the ionic dissociation constant of dinitrogen tetroxide. It is $K < 1{\cdot}9 \times 10^{-3}$ mole l.$^{-1}$, consistently with our previous knowledge that dinitrogen tetroxide, though a strong electrolyte in nitric acid, becomes a weak electrolyte, obeying Ostwald's dilution law, in mixtures of nitric acid with organic solvents.

Thirdly, using the above upper limit of K, and the determined value of $k_2''K$, we can derive a lower limit for k_2'', the rate constant for nitrosation by the nitrosonium ion. The value is below, and it is of interest to compare it with the rate constant for nitrosation by dinitrogen tetroxide :

$$k_2'' > 132 \times 10^{-3} \text{ sec.}^{-1} \text{ mole}^{-1} \text{ l.} \quad k_2' = 13 \times 10^{-3} \text{ sec.}^{-1} \text{ mole}^{-1} \text{ l.}$$

The figures show that the nitrosonium ion is specifically more than ten times faster as a reagent for nitrosation than is the dinitrogen tetroxide molecule. The actual factor may be much greater than ten.

Experimental

Materials.—The nitrating mixtures were made from absolute nitric acid, acetic acid, and dinitrogen tetroxide, each purified as directed in Part II (*J.*, 1950, 2400). Solutions containing suitable amounts of stoicheiometric nitrous acid were prepared by mixing a solution free from nitrous acid with a solution to which freshly distilled dinitrogen tetroxide had been added. The *p*-chloroanisole was obtained as directed in Part VI (*J.*, 1950, 2628). Tetramethylammonium nitrate (Found by use of Devarda's alloy : NO_3^-, 46·0. Calc. for $C_4H_{12}N{\cdot}NO_3$: NO_3^-, 45·5%) and tetraethylammonium nitrate (Found : NO_3^-, 31·9. Calc. for $C_8H_{20}N{\cdot}NO_3$: NO_3^-, 32·2%) were prepared by way of the iodides and hydroxides, and after crystallisation were carefully dried.

Kinetics.—The dilatometric method was employed, using tapless dilatometers, as in Part VI. The first-order rate law, checked graphically, was well obeyed by the individual runs. Best values of first-order rate constants were calculated by Guggenheim's pairing method. During preparation for a set of runs, including the final series reported in the table, a bulk solution of nitric acid in acetic acid was made up, in order to secure constancy in the concentration of nitric acid. But it was not found possible similarly to standardise the concentrations of nitrous acid, and therefore this was introduced (as dinitrogen tetroxide) and analytically determined on the occasion of each run. It was determined both before and after each run, always with substantially identical results.

Analysis.—The dilatometric indication of reaction progress had already been checked for this nitration by chemical analysis (Part VI). Concentrations of nitrous acid were estimated by the colorimetric method (Part II).

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9. *An Ultrafiltration Method for the Optical Clarification of Protein Solutions in Light-scattering Investigations.*

By D. A. I. Goring and P. Johnson.

In the application of the light-scattering method to molecular-weight and -size determinations of dissolved macromolecules, removal of aggregated material and all impurity is essential. The more usual methods of filtration are inadequate and a simple ultrafiltration technique based on Elford's Gradacol membranes has been evolved. By its use molecular-weight values in fair agreement with accepted values have been obtained as well as low and reproducible dissymmetry values.

Since the light-scattering method yields a weight-average molecular weight (M) for dissolved macromolecules, erroneously large values are caused by the presence of relatively small amounts of heavy aggregates or impurity. In a current light-scattering investigation considerable difficulty was found in removing such aggregates from solutions of certain globulin-type seed proteins. For aqueous systems the ordinary filtration methods employing fine sintered discs, Seitz or cellulose-pulp pads were ineffective; high-speed centrifugation (12 000 r.p.m.) only partially cleared the solutions, for it was found impossible not to stir up sediment when removing tubes from the rotor. An ultrafiltration technique based on the Gradacol membranes developed by Elford (*Trans. Faraday Soc.*, 1937, **33**, 1094) was finally adopted, with which clear solutions were obtained, giving molecular weights near the accepted values. The light-scattering apparatus used is described elsewhere (Goring and Johnson, publication pending).

Experimental

Membranes were made in the same way as the thimble-type osmometer membranes described by Adair (*Proc. Roy. Soc.*, 1925, *A*, **108**, 627) and Alexander and Johnson ("Colloid Science," Oxford Univ. Press, 1949). A suitable nitrocellulose solution was poured evenly over a test tube-shaped mould perforated by a small hole at the rounded end; the mould was rotated at about 15 r.p.m. Each coat was allowed to dry for a given time before the next was applied. No heat was used for drying, and the temperature and humidity were not controlled. With Elford's Gradacol membranes, intended for gradation of particle size, reproducible and uniform pore diameters are ensured by strict control of temperature and humidity in the drying process. Since the purpose here was merely removal of aggregates, such care in preparation was considered unnecessary. After soaking in water overnight the thimble was removed by forcing water under pressure between the film and the glass through the perforation. High quality rubber pressure tubing was used for mounting and the final size of the membrane was *ca.* 40 mm. long by 10 mm. diameter, giving a filter area of 12 sq. cm. Membranes were stored in 40% saturated aqueous ammonium sulphate and were cleaned for re-use with N/10-hydrochloric acid or 0·1% sodium hydroxide solution. More concentrated solutions of alkali caused the nitrocellulose to become brittle and crack.

Absolute ethanol, sodium-dried ether, and AnalaR acetone, amyl alcohol, and glacial acetic acid were used as solvents; the nitrocellulose was a sample (grade HL 120/170) recommended by Alexander and Johnson for membrane formation. The most useful membranes for filtration of seed-globulin solutions ($M \approx 300{,}000$) were made by applying three coats of a solution of the following composition: nitrocellulose (2 g.), ether (18 g.), alcohol (7 g.), acetone (9 g.), amyl alcohol (4 g.), and acetic acid (0·13 g.).

As reported by Elford the pore size was found to be sensitive to the concentration of acetic acid, and membranes with greater or smaller permeabilities were made by using, respectively, less or more acetic acid in the nitrocellulose solution. Times of drying before the second and the third coat were applied were 2 and 3 minutes, respectively, and, for the final drying, moulds were suspended in a vertical position for 1 hour before being soaked. The permeability of these membranes to water was 0·7 c.c./minute under a pressure of 5 cm. of mercury and was fairly reproducible ($\pm 10\%$). With protein solutions, flow rates were slower because of the increased viscosity of the fluid and probably the blockage of the pores by large aggregates.

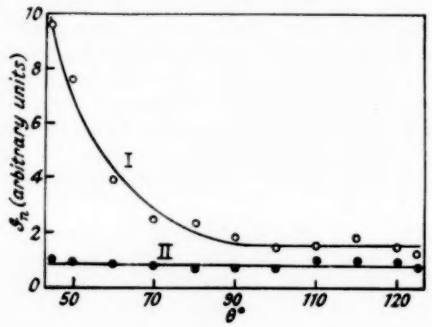
By means of a mercury manometer, protein solutions were forced through the membranes

under pressures of 5—15 cm. Hg. If the solution was so cloudy that filtration proved very slow, a preliminary filtration through a sintered glass disc was found preferable to the use of greater pressures which tended to pollute the solution. The proportion of material held up by the filter varied with the protein preparation, the state of the solution, and the ionic strength, I. With serum albumin ($I = 0{\cdot}1$) and edestin solutions (from hemp seed) ($I = 1$), respectively, the hold-up of material was 15% and 25%, while for strongly aggregated solutions of arachin (from ground-nuts) ($I = 0{\cdot}1$) less than half of the protein was found in the filtrate.

Easy filtration with small hold-up was favoured by solutions of high ionic strength. For example, with a solution of legumin (from peas) in phosphate buffer of pH 7·7 and $I = 0{\cdot}1$, the hold-up was 75%, falling to 20% when the ionic strength was increased to 0·5. This effect may have been due to either the increasing tendency to aggregation at low values of I (due to the lowered solubility of the globulin), or an increase in particle–membrane interaction at low ionic strengths.

An example of the effectiveness of the filters is given in the figure which shows the normalised intensity distributions of scattered light for buffers cleaned, respectively, by filtration through

Plot of $\mathscr{I}_n$ against θ for buffer solutions filtered through a No. 4 " Pyrex " sintered-glass disc (I) *and an ultrafiltration membrane* (II).



a No. 4. Pyrex sintered-glass disc, and by the present ultrafiltration technique. The normalised intensity $\mathscr{I}_n$ is given by :

$$\mathscr{I}_n = \mathscr{I} \sin\theta/(1 + \cos^2\theta)$$

where $\mathscr{I}$ is the observed intensity and θ is the angle the scattered light makes with the transmitted beam. For the ultra-filtered buffer, $\mathscr{I}_n$ is constant and the dissymmetry, $\mathscr{I}_{60°}/\mathscr{I}_{120°}$, is virtually unity, indicating that heavy material had been completely removed by the filter. The irregularities in the graph are due to small imperfections in the light-scattering apparatus which become apparent at such low values of $\mathscr{I}_n$; here $\mathscr{I}_n$ for the ultra-filtered buffer is less than 1% of the scatter shown by a 0·3% solution of arachin. With the buffer filtered by the sintered-glass disc, $\mathscr{I}_n$ increased markedly for smaller values of θ, and $\mathscr{I}_{60°}/\mathscr{I}_{120°}$ was 2·7, showing the presence of large scattering particles in the buffer.

As a further test, solutions of serum albumin and arachin, known to contain a considerable amount of aggregated material, were filtered first through a No. 4. " Pyrex " sintered-glass disc and then through a membrane. Light-scattering determinations of molecular weight were made after each filtration. From the results of these measurements, shown in the Table, it is

***Decrease in** Molecular Weight and Dissymmetry after Ultrafiltration.*

	M			$\mathscr{I}_{60}/\mathscr{I}_{120}$	
Protein	Accepted value	found, before ultrafiltration	found, after ultrafiltration	before ultrafiltration	after ultrafiltration
Arachin	330,000	3,260,000	542,000	1·95	1·26
Serum albumin	70,000	140,000	85,000	1·69	1·04

clear that much of the aggregated material passed through the sintered-glass disc to give high values of M and $\mathscr{I}_{60}/\mathscr{I}_{120}$ which were considerably reduced after ultrafiltration. The M values were still somewhat higher than the accepted molecular weights because strongly aggregated

solutions were especially chosen to test the membranes. Freshly prepared solutions gave accurate values for the molecular weight of serum albumin; for the seed globulins, arachin, legumin, and edestin, molecular weights only 10% higher than the accepted values were noted. This discrepancy is not necessarily attributed entirely to the light-scattering determination.

We are grateful to Mr. B. P. Brand for the preparation of the legumin samples used.

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10. *The Reaction of Some Sugar Acetates with Ammonia.*

By R. ALLERTON and W. G. OVEREND.

The reaction of *aldehydo*-sugar acetates with ammonia is discussed.

ISBELL and FRUSH (*J. Amer. Chem. Soc.*, 1949, **71**, 1579) showed that reaction of *aldehydo*-L-arabinose tetra-acetate with ammonia in methanol or with concentrated aqueous ammonia gives *NN*-diacetyl-L-arabinosylamine. A mechanism was suggested to account for the formation of this derivative. This postulated the addition of ammonia to the *aldehydo*-form of the sugar, followed by migration of a neighbouring acetyl group to the amino-nitrogen by way of an intermediate ortho-ester (cf. Fischer, *Ber.*, 1920, **53**, 1621; Hibbert *et al.*, *J. Amer. Chem. Soc.*, 1929, **51**, 1601; *Canad. J. Res.*, 1931, **4**, 254; Farmer, *Ann. Reports*, 1930, **27**, 103; see Hirst and Peat, *ibid.*, 1934, **31**, 172, for evidence of the mechanism). The diacetamido-derivative could be formed from the monoacetamido-derivative by replacement of the hemiacetal hydroxyl group with ammonia, followed by a further migration of the neighbouring acetyl group (cf. Hockett, Deulofeu, and Defarrari, *J. Amer. Chem. Soc.*, 1950, **72**, 1840; cf. Deulofeu and Defarrari, *Nature*, 1951, **167**, 42).

The reaction seems to be general since it has been shown in this department in collaboration with Dr. A. S. Jones that treatment of *aldehydo*-D-galactose penta-acetate with methanolic ammonia results in the formation of *NN*-diacetyl-D-galactosylamine which can be characterised as its penta-acetate. However, it has been demonstrated that the migration of the acetyl groups to $C_{(1)}$ occurs only from $C_{(2)}$ or $C_{(3)}$. There is no migration, for instance, if the hydroxyl groups at $C_{(2)}$ and $C_{(3)}$ are blocked by other residues. 2 : 3-Dimethyl *aldehydo*-D-glucose 4 : 5 : 6-triacetate was prepared from 2 : 3-dimethyl D-glucose (cf. *Canad. J. Res.*, 1942, **20**, *B*, 175) by the usual sequence of reactions (cf. Wolfrom, *J. Amer. Chem. Soc.*, 1930, **52**, 2464) involving the formation of 2 : 3-dimethyl D-glucose diethyl mercaptal and its triacetate. Removal of sulphur from the latter compound afforded 2 : 3-dimethyl *aldehydo*-D-glucose 4 : 5 : 6-triacetate. Treatment of this with methanolic ammonia gave a nitrogen-free product shown to be 2 : 3-dimethyl D-glucose chromatographically and by preparation of the diethyl mercaptal and *p*-toluidide. No trace of a mono- or di-acetamido-derivative of 2 : 3-dimethyl D-glucose was detected.

EXPERIMENTAL

NN-Diacetyl-D-galactosylamine 2 : 3 : 4 : 5 : 6-penta-acetate. *aldehydo*-D-Galactose penta-acetate (1 g.; Wolfrom, *J. Amer. Chem. Soc.*, 1930, **52**, 2464) was dissolved in dry methanol (40 c.c.), and the solution was saturated at 0° with gaseous ammonia. After being kept at 0° for 18 hours the solution was evaporated to dryness. Ammonium acetate was removed from the residue at 70—80°/0·005 mm. The gelatinous product, dissolved in dry pyridine (6 c.c.), was cooled at −5°. Freshly distilled acetic anhydride (3 c.c.) was added and the mixture was kept at 0° for 24 hours and then for a further 24 hours at room temperature. After being poured into water, the mixture was extracted with chloroform. The extract was washed with water, dried ($MgSO_4$), and evaporated to dryness. The residue was kept *in vacuo* over concentrated sulphuric acid and phosphoric oxide until quite dry. Thereafter it was dissolved in warm acetic acid and passed through a small column of alumina. On addition

of light petroleum to the eluate, needles separated. Recrystallisation from acetic acid–light petroleum gave NN-*diacetyl*-D-*galactosylamine penta-acetate* (0·3 g.) as colourles needles, m. p. 205°, $[\alpha]_D^{19}$ −38·9° (*c*, 1·0 in chloroform) (Found: C, 48·3; H, 6·2; N, 5·5. $C_{20}H_{30}O_{12}N_2$ requires C, 48·8; H, 6·2; N, 5·7%).

2 : 3-*Dimethyl* D-*glucose* p-*toluidide*. 2 : 3-Dimethyl D-glucose (0·5 g.) (Freudenberg, Toepffer, and Andersen, *Ber.*, 1928, **61**, 1750) was boiled under reflux for 3 hours with *p*-toluidine (0·5 g.) in absolute ethanol (100 c.c.). Evaporation to dryness then afforded a syrup which slowly crystallised at 0°. Recrystallisation from aqueous ethanol afforded the *p*-toluidide as long colourless needles, m. p. 151°, $[\alpha]_D^{21}$ +26·6° (equilibrium) (*c*, 1·13 in ethanol) (Found: C, 61·0; H, 7·1. Calc. for $C_{15}H_{23}O_5N$: C, 60·6; H, 7·7%).

2 : 3-*Dimethyl* D-*glucose diethyl mercaptal*. 2 : 3-Dimethyl D-glucose (6·04 g.) was shaken for 4·5 hours with ethanethiol (4 c.c.) and concentrated hydrochloric acid (4 c.c.). After being poured into water, the mixture was neutralised by addition of sodium carbonate, and then concentrated to dryness. The solid residue was extracted with hot ethanol. Removal of the solvent yielded a syrup. On distillation (b. p. 190—220°/0·05 mm.) the *diethyl mercaptal* (4·7 g.) was obtained as a yellow viscous syrup, $[\alpha]_D^{20}$ +35·3° (*c*, 0·68 in chloroform) (Found: C, 45·4; H, 8·6. $C_{12}H_{26}O_5S_2$ requires C, 45·8; H, 8·3%). This (4·48 g.) with acetic anhydride (8 c.c.) in dry pyridine (20 c.c.) gave 4 : 5 : 6-*triacetyl* 2 : 3-*dimethyl* D-*glucose diethyl mercaptal* (4·0 g.) as a syrup, b. p. 175—190°/0·05 mm., n_D^{24} 1·4728, $[\alpha]_D^{19}$ +37·6°, +38·8° (*c*, 1·11, 2·06 in chloroform) (Found: C, 49·7; H, 7·2. $C_{18}H_{32}O_8S_2$ requires C, 49·1; H, 7·3%).

4 : 5 : 6-*Triacetyl* 2 : 3-*dimethyl* aldehydo-D-*glucose*. 4 : 5 : 6-Triacetyl 2 : 3-dimethyl D-glucose diethyl mercaptal (4·91 g.) was dissolved in acetone (22·6 c.c.) and water (11·3 c.c.). Cadmium carbonate (15·4 g.) was added to the well-stirred solution and then slowly a solution of mercuric chloride (12·1 g.) in acetone (18 c.c.). The mixture was stirred at room temperature for 24 hours, then filtered on to a small amount of cadmium carbonate. The residue was washed several times with acetone and the combined filtrate and washings were evaporated to dryness in the presence of some cadmium carbonate. The residue was dried by distilling ethanol over it, and then extracted with hot chloroform (3 × 15 c.c.). The chloroform extract was washed with aqueous potassium iodide and then water (4 × 15 c.c.), and dried ($MgSO_4$). After passage through charcoal the solution was evaporated and the syrupy residue was distilled. 4 : 5 : 6-*Triacetyl* 2 : 3-*dimethyl* aldehydo-D-*glucose* (2·5 g.) was obtained as a colourless syrup, b. p. 135—137°/0·02 mm., n_D^{21} 1·4591, $[\alpha]_D^{18}$ +52·4° (*c*, 1·1 in chloroform), $\lambda_{max.}$ 290 mμ, ε, 271 (Found.: C, 49·9; H, 6·7; OMe, 18·7. $C_{14}H_{22}O_9$ requires C, 50·4; H, 6·6; OMe, 18·6%). This syrup strongly reduced Fehling's solution.

Action of methanolic ammonia on 4 : 5 : 6-*triacetyl* 2 : 3-*dimethyl* aldehydo-D-*glucose*. A solution of 2 : 3-dimethyl 4 : 5 : 6-triacetyl *aldehydo*-D-glucose (1·88 g.) in dry methanol (100 c.c.) was saturated at 0° with ammonia. The mixture was kept at 0° for 18 hours and then filtered. The filtrate was evaporated at 35°, and the syrupy residue thoroughly dried and then extracted with hot ethyl acetate, ammonium acetate remaining undissolved. After passage through charcoal the ethyl acetate solution was concentrated to dryness and afforded a syrup which was strongly reducing and slowly crystallised on storage *in vacuo*. It then had m. p. 85—90°; Irvine and Scott (*J.*, 1913, **103**, 575) give 85—87° for the α-form and m. p. 121° for the β-form. McClosky and Coleman (*J. Org. Chem.*, 1945, **10**, 184) report m. p. 110° for 2 : 3-dimethyl β-D-glucose. The product was 2 : 3-dimethyl D-glucose since it readily gave 2 : 3-dimethyl D-glucose diethyl mercaptal, b. p. 190—220°/0·02 mm., $[\alpha]_D^{17}$ +33·2° (*c*, 0·48 in chloroform), and the *p*-toluidide, m. p. 151° alone or mixed with a sample prepared as above. 1% Aqueous solutions of the syrupy and the crystalline 2 : 3-dimethyl αβ-D-glucose were run on paper chromatograms with butanol–ethanol–ammonia as eluent (cf. Allerton and Overend, *J.*, 1951, 1480), with 2 : 3-dimethyl D-glucose as a reference compound. The chromatographic behaviour of the samples was identical.

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11. *Anhydrides of Polyhydric Alcohols. Part XVI.* The Action of Phenols on Some Ethylene Oxide Derivatives.*

By G. P. McSweeney, L. F. Wiggins, and D. J. C. Wood.

A study has been made of the action of certain phenols on 1 : 2-5 : 6-diepoxyhexane, 3 : 4-*iso*propylidene 1 : 2-5 : 6-dianhydromannitol, and 1 : 3-2 : 4-diethylidene 5 : 6-anhydrosorbitol. The products obtained are designated as 1 : 6-diaryloxyhexane-2 : 5-diol, 1 : 6-diaryl 3 : 4-*iso*propylidene mannitol, and 6-aryl 1 : 3-2 : 4-diethylidene sorbitol respectively. The phenyl groups are attached to the primary carbon atoms since, when propylene oxide is treated in an exactly similar manner, the product is proved, by an alternative synthesis, to be 1-phenoxypropan-2-ol. The product of the action of phenol on 3 : 4-*iso*propylidene 1 : 2-5 : 6-dianhydromannitol is synthesized by an alternative route which indicates that it is 1 : 6-diphenyl 3 : 4-*iso*propylidene mannitol.

Earlier communications of this series have recorded some reactions of 1 : 2-5 : 6-diepoxyhexane (I), 3 : 4-*iso*propylidene 1 : 2-5 : 6-dianhydromannitol (II) (*J.*, 1950, 1566; 1946, 384), and 1 : 3-2 : 4-diethylidene 5 : 6-anhydrosorbitol (III) (*J.*, 1946, 388). This work has now been extended to a study of the behaviour of these ethylene oxide derivatives towards various phenols.

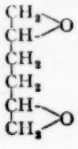
It is known that ring fission of ethylene oxide ring compounds by phenols may be brought about under a variety of conditions. For example, a slow combination occurs when phenols and ethylene oxide itself are heated under pressure (Roithner, *Monatsh.*, 1894, **15**, 665); reaction is more rapid in the presence of an alkali-metal derivative of the phenol in a solvent such as water or alcohol (Boyd and Marle, *J.*, 1910, 1788; 1914, 2117). In the present work the reactions were effected by heating the appropriate phenol with the epoxy-compound in dry benzene solution in the presence of a suspension of the sodium derivative of the phenol, usually for about 7 hours. The products were isolated by addition of water and in most cases were crystalline.

The treatment of 1 : 2-5 : 6-diepoxyhexane (I) with phenol led to a crystalline product which was separated by fractional recrystallisation into approximately equal amounts of two diphenoxyhexanediols. Each isomeride gave rise to crystalline diacetyl and ditoluene-*p*-sulphonyl derivatives. *p*-Cresol and (I) also gave a mixture of two solid di-*p*-tolyloxyhexanediols, each of which gave a crystalline diacetate. With *m*-cresol, the reaction similarly led to two solid isomerides. In each of these three cases, the melting points of the pairs of isomerides were found to differ by about 30°. When the reaction with diepoxyhexane was carried out with *o*-cresol, only one solid di-*o*-tolyloxyhexanediol could be obtained.

In the earlier communication on the reactions of 1 : 2-5 : 6-diepoxyhexane (Part XIV, *J.*, 1950, 1566), theoretical considerations were shown to suggest that two isomeric forms of the epoxide should exist and the experimental results described supported this idea. The existence of isomerism in the products of the reaction of diepoxyhexane with phenols is in conformity with this view and it would accordingly be expected that, of the two isomerides obtained, one would be a *meso*- or *cis*-form while the other would be a racemic mixture of the corresponding D- and L- or *trans*-forms. If the aryloxy-groups are assumed to be attached to the primary carbon atoms of the hexane chain, the two forms of the condensation product from phenol, for example, would be represented by the 1 : 6-diphenoxyhexane-2 : 5-diols (IV) and (V). This assumption appears to be justified by the fact that, in all our previous work on diepoxyhexane involving its ring fission under conditions similar to those used here, no evidence was found to suggest that ring opening occurred in any manner other than that leading to combination of the anionic part of the

* Part XV, *J.*, 1951, 1180.

reagent molecule with the primary carbon atoms. Nevertheless, the possibility of ring fission occurring in the reverse direction, giving isomeric 2 : 5-diphenoxyhexane-1 : 6-diol, cannot be completely dismissed and in an attempt to decide the question the ditoluene-*p*-sulphonates of the two diphenoxyhexanediols were each treated with sodium iodide and acetone at 100° according to Oldham and Rutherford's method (*J. Amer. Chem. Soc.*, 1932, **54**, 366) for distinguishing between primary and secondary hydroxyl groups. It was expected that no reaction would occur in either case, but, in fact, both toluene-*p*-sulphonates reacted readily to give a high yield of sodium toluene-*p*-sulphonate.



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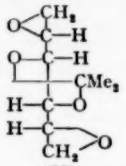

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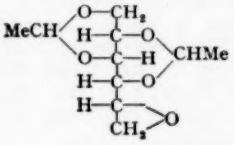

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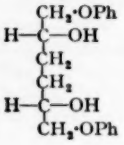

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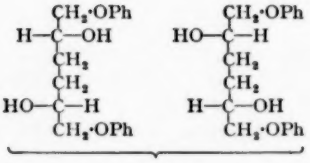

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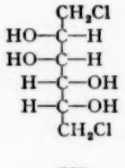

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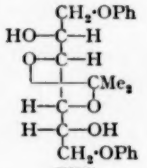

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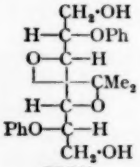

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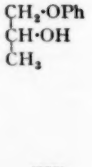

(IX)

In view of the exceptions observed to Oldham and Rutherford's rule, it was thought desirable to seek further evidence. This was obtained by a study of the action of phenol on 3 : 4-*iso*propylidene 1 : 2-5 : 6-dianhydromannitol (II) under the same conditions as those employed with diepoxyhexane. A crystalline compound was obtained which ultimate analysis showed to be a diphenyl *iso*propylidene hexitol. The same compound was obtained by an alternative route, namely, the condensation of a di*iso*propylidene derivative of 1 : 6-dichloro-1 : 6-dideoxymannitol (VI) with sodium phenoxide in acetone. Further proof of the identity of the two specimens of this compound was supplied by the acid hydrolysis of each to give the same 1 : 6-diphenyl mannitol, benzoylation of which yielded 2 : 3 : 4 : 5-tetrabenzoyl 1 : 6-diphenyl mannitol. During this reaction an unexpected complication arose inasmuch as one of the *iso*propylidene groups attached to the mannitol molecule became detached and it is not possible to state, at present, a reason for this. The chlorine atoms in the starting material were known to be attached to $C_{(1)}$ and $C_{(6)}$ by reason of the preparation of the compound from 1 : 6-dichloro-1 : 6-dideoxymannitol (Wiggins, *J.*, 1946, 384), and it therefore follows that the phenoxy-groups in the condensation product should also be at positions 1 and 6. However, because it is not known how the one *iso*propylidene residue from the original di*iso*propylidene 1 : 6-dichloro-1 : 6-dideoxymannitol became dislodged, this cannot be regarded as a rigid proof for the allocation of the phenol residues to $C_{(1)}$ and $C_{(6)}$. Nevertheless, the result obtained above very strongly suggests that the diphenyl *iso*propylidene hexitol, whether obtained from the dichloro-compound or from *iso*propylidene dianhydromannitol is 1 : 6-diphenyl 3 : 4-*iso*propylidene mannitol (VII). Ring fission of the dianhydride in the reverse direction would imply the probable occurrence of Walden inversion on $C_{(2)}$ and $C_{(5)}$ (these atoms becoming transitional carbonium cations), which would lead to the formation of a L-iditol derivative (VIII).

Therefore it is most probable that the action of phenol on 3 : 4-*iso*propylidene 1 : 2-5 : 6-dianhydromannitol results in ring fission at the bonds between the oxiran-oxygen atoms and the primary carbon atoms with the resultant attachment of phenoxy-groups to the latter. By analogy, these observations provide strong support for the idea that ring fission of 1 : 2-5 : 6-diepoxyhexane by phenol occurs in a similar manner, giving 1 : 6-diphenoxyhexane-2 : 5-diol.

Still further evidence in support of this contention has been obtained by studying the reaction of propylene oxide with phenol in benzene in the presence of sodium phenoxide under the conditions used for the reaction with 1 : 2-5 : 6-diepoxyhexane. The product was a liquid which showed the properties recorded by Hurd and Perlitz (*J. Amer. Chem. Soc.*, 1946, **68**, 38) for 1-phenoxypropan-2-ol (IX). It yielded a crystalline toluene-*p*-sulphonate. Its structure was proved by its synthesis from methylmagnesium iodide and phenoxyacetaldehyde, which can lead only to 1-phenoxypropan-2-ol. The crystalline toluene-*p*-sulphonate was identical with that obtained from the product of reaction of propylene oxide with phenol. This shows conclusively that the reaction between phenol and propylene oxide leads to the addition of the phenoxy-group at the primary carbon atom. It is reasonable to assume that this takes place also in the condensation of phenols with 1 : 2-5 : 6-diepoxyhexane and 3 : 4-*iso*propylidene 1 : 2-5 : 6-dianhydromannitol and that the products of these reactions are therefore 1 : 6-diaryloxyhexane-2 : 5-diols and 1 : 6-diaryl 3 : 4-*iso*propylidene mannitol respectively.

In an attempt to explain the reactivity of the ditoluene-*p*-sulphonyl derivatives of the two isomeric diphenoxyhexanediols towards sodium iodide, the behaviour, under the same conditions, of the ditoluene-*p*-sulphonate of 1 : 6-diphenyl 3 : 4-*iso*propylidene mannitol (VII) and of the toluene-*p*-sulphonate of 1-phenoxypropan-2-ol was examined. Reaction of (VII) with toluene-*p*-sulphonyl chloride in pyridine solution did not proceed at room temperature but was effected by prolonged heating of the mixture at 130°; doubtless there is steric hindrance by the phenyl and the *iso*propylidene groups. The ditoluene-*p*-sulphonate did not react with sodium iodide in acetone, as might be expected, but the same was true after the *iso*propylidene group, and hence the possible steric hindrance, had been removed by acid hydrolysis. 1-Phenoxyprop-2-yl toluene-*p*-sulphonate, which indisputably contains a toluene-*p*-sulphonyl residue attached to a secondary carbon atom, reacted readily with sodium iodide in acetone solution and furnished 0·92 mol. of sodium toluene-*p*-sulphonate. It appears that the difference in the behaviour of the toluene-*p*-sulphonates of, on the one hand, diphenoxyhexanediol and 1-phenoxypropan-2-ol and, on the other, diphenyl *iso*propylidene mannitol must be ascribed to the presence of the deoxy-groupings at $C_{(3)}$ and $C_{(4)}$ in the hexane derivative and at $C_{(3)}$ in the propane derivative.

3 : 4-*iso*Propylidene 1 : 2-5 : 6-dianhydromannitol was also treated with *p*-chlorophenol and with *m*-cresol. The two crystalline products obtained were 1 : 6-di-*p*-chlorophenyl 3 : 4-*iso*propylidene mannitol and 3 : 4-*iso*propylidene 1 : 6-di-*m*-tolyl mannitol respectively. Acid hydrolysis of these afforded 1 : 6-di-*p*-chlorophenyl and 1 : 6-di-*m*-tolyl mannitol.

When 1 : 3-2 : 4-diethylidene 5 : 6-anhydrosorbitol (III) was treated under similar conditions with phenol and *o*- and *m*-cresol, three crystalline products were isolated which, by analogy, are the 6-phenyl and 6-*o*- and 6-*m*-tolyl derivatives of 1 : 3-2 : 4-diethylidene sorbitol. Hydrolysis of the *o*- or *m*-tolyl compound yielded syrupy 6-tolyl sorbitols, characterised as their crystalline penta-acetates. 1 : 3-2 : 4-Diethylidene 6-*o*-tolyl sorbitol yielded a crystalline toluene-*p*-sulphonate which did not react with sodium iodide in acetone at 105°.

Although the study of the reaction of ethylene oxide compounds, particularly those containing two epoxy-rings, with phenols was of interest from the theoretical viewpoint, this was not the main reason for undertaking this work. It has been shown (Berger and Bradley, *Brit. J. Pharmacol.*, 1946, **1**, 265) that certain aryl ethers of glycerol, particularly the *o*-tolyl ethers (3-*o*-tolyloxypropane-1 : 2-diol, "Myanesin"), show muscle-relaxing activity, and the aryl ethers of mannitol and sorbitol described above were prepared in order that their pharmacological activity might be examined. This phase of the work is now being studied by others and the results will be published elsewhere.

Experimental

Action of Phenol on 1 : 2-5 : 6-Diepoxyhexane.—To a solution of phenol (5 g.) in benzene (20 c.c.), in which sodium (0·2 g.) had been dissolved, diepoxyhexane (1·2 g.) was added, and the mixture heated on the boiling-water bath for 7 hours. Water was then added to the cooled mixture, and the white crystalline solid which separated, collected, and washed with dilute sodium

hydroxide solution and with water. The crude product (2·3 g.), after being once recrystallized from alcohol, gave fine, feathery needles (1·3 g.), m. p. 135—143°. Fractional recrystallization from a large volume of alcohol effected separation of this material into two components : colourless plates (0·24 g.) of 1 : 6-*diphenoxyhexane*-2 : 5-*diol*, m. p. 163—164·5° (Found : C, 71·3; H, 7·35. $C_{18}H_{22}O_4$ requires C, 71·5; H, 7·3%); and stout needles (0·16 g.), m. p. 135—136·5°, of an *isomer* (Found : C, 71·7; H, 7·3%).

The diol of m. p. 163—164·5° (0·09 g.) and acetic anhydride (0·5 c.c.) in dry pyridine (2 c.c.) were kept at about 30° for 24 hours, then poured into water; the solid *diacetate* produced was collected, washed with water, and recrystallized from alcohol in needles (0·09 g.), m. p. 102—103° (Found : C, 68·1; H, 6·7. $C_{22}H_{26}O_6$ requires C, 68·4; H, 6·7%).

The diol of m. p. 135—136·5° similarly gave a *diacetate* (0·10 g.) [from light petroleum (b. p. 60—80°)] as prisms, m. p. 70—71·5° (Found : C, 68·4; H, 6·8%).

The diol of m. p. 163—164·5° (0·09 g.) and toluene-*p*-sulphonyl chloride (0·24 g.) in dry pyridine (5 c.c.), first at 0° and then at about 30° (overnight), gave the *ditoluene*-p-*sulphonate*, m. p. 177—178° (decomp.), as needles from chloroform (Found : C, 62·6; H, 5·7. $C_{32}H_{34}O_8S_2$ requires C, 63·0; H, 5·6%). The derivative (0·057 g.) was heated with dry sodium iodide (0·25 g.) and dry acetone (10 c.c.) in a sealed tube at 100° for 7 hours. The precipitate of sodium toluene-*p*-sulphonate which rapidly formed was collected, washed with a little dry acetone and dried at 110°; the yield was 0·032 g. (1·78 equivs.)

The isomeric diol (0·12 g.) similarly gave a *ditoluene*-p-*sulphonate* as needles (0·1 g.), m. p. 124—125° (from alcohol) (Found : C, 63·0; H, 5·6%). With dry sodium iodide (0·4 g.) and dry acetone (10 c.c.) in a sealed tube at 100° for 8 hours, it rapidly gave sodium toluene-*p*-sulphonate (0·057 g., 2·0 equivs.).

Action of o-*Cresol on* 1 : 2-5 : 6-*Diepoxyhexane*.—A solution of *o*-cresol (2·5 g.) and sodium (0·1 g.) in dry benzene (10 c.c.) was treated with diepoxyhexane (0·5 g.) and the whole heated for 7 hours. Water was added, the benzene layer separated, and the aqueous layer extracted with benzene. The combined extracts were dried ($MgSO_4$) and evaporated to a syrup (1·1 g.) which partly crystallized. After addition of ether and light petroleum, the crystalline material (0·33 g.) was collected and recrystallized from alcohol, forming prisms of 1 : 6-*di*-o-*tolyloxyhexane*-2 : 5-*diol*, m. p. 95—97° (Found : C, 72·7; H, 7·7. $C_{20}H_{26}O_4$ requires C, 72·8; H, 7·9%)

Action of m-*Cresol on* 1 : 2-5 : 6-*Diepoxyhexane*.—Diepoxyhexane was treated with *m*-cresol as described for *o*-cresol. When heating of the reaction mixture was complete, dilute sodium hydroxide solution was added and the separated solid collected, washed free from cresol, and recrystallized from aqueous alcohol, to give clusters of fine needles (0·4 g.), m. p. 95—106°. The benzene layer from the reaction mixture was evaporated to a solid, which, recrystallized from alcohol, formed shining plates (0·11 g.), m. p. 93·5—94·5°. It was 1 : 6-*di*-m-*tolyloxyhexane*-2 : 5-*diol* (Found : C, 72·5; H, 8·15%). Fractional recrystallization of the product of m. p. 95—106° yielded a first fraction (0·02 g.) which formed needles, m. p. 119—120°, and was the isomeric *diol* (Found : C, 72·8; H, 8·3%), and a final fraction (0·03 g.) which crystallized in plates, m. p. 92—94°, and was identical with the compound, m. p. 93·5—94·5°, isolated from the benzene layer.

Action of p-*Cresol on* 1 : 2-5 : 6-*Diepoxyhexane*.—The previous experiment was repeated using *p*-cresol. The crude condensation product crystallized from alcohol in feathery needles (0·4 g.), m. p. 143—155°. Fractional recrystallization from alcohol separated the compound into two isomeric 1 : 6-*di*-p-*tolyloxyhexane*-2 : 5-*diols*; the first fraction (0·025 g.) formed plates, m. p. 173—174·5° (Found : C, 73·2; H, 7·6%), and the final fraction small prisms (0·043 g.), m. p. 143·5—145° (Found : C, 72·5; H, 8·0%).

The mixture (0·20 g.) of m. p. 143—145° was heated with acetic anhydride (0·5 c.c.) and dry pyridine (2·5 c.c.) on a boiling-water bath for 2½ hours. The product, isolated by addition of water, formed small nodules (0·19 g.), m. p. 92—120°, from alcohol. Recrystallization from alcohol yielded two isomeric *diacetates*, the first fraction forming needles (0·04 g.), m. p. 49·5—51° (Found : C, 69·3; H, 7·0%), and the final fraction prismatic needles (0·03 g.), m. p. 123—125° (Found : C, 69·4; H, 7·1%).

Action of Phenol on 3 : 4-iso*Propylidene* 1 : 2-5 : 6-*Dianhydromannitol*.—A solution of sodium (0·25 g.) and phenol (5 g.) in dry benzene (20 c.c.) was treated with *iso*propylidene dianhydromannitol (1 g.) and the whole heated for 5 hours. After the mixture had cooled, water (20 c.c.) was added and the benzene layer diluted with twice its volume of light petroleum. The separated solid was collected and washed successively with sodium hydroxide solution and water. Recrystallization from light petroleum (b.p. 100—120°) gave 1 : 6-*diphenyl* 3 : 4-iso-

propylidene mannitol (1·9 g.) in long colourless needles, m. p. 115° (Found : C, 67·5; H, 6·6. $C_{21}H_{36}O_6$ requires C, 67·5; H, 6·9%).

This product (0·1 g.) was heated with 0·1N-sulphuric acid (25 c.c.) and alcohol (5 c.c.) on the boiling-water bath for 3 hours. The solid (0·08 g.) which separated was collected, washed with water, and recrystallized from acetone, forming 1 : 6-*diphenyl mannitol* in small plates, m. p. 200—204° (Found : C, 64·9; H, 6·7. $C_{18}H_{22}O_6$ requires C, 64·7; H, 6·6%). This (0·04 g.) was dissolved in dry pyridine (2 c.c.) and benzoyl chloride (0·5 c.c.) added. After being kept at about 30° for 48 hours, the solution was poured into water and extracted with chloroform; the extract was washed successively with dilute sulphuric acid, sodium hydrogen carbonate solution, and water, dried ($MgSO_4$), and evaporated to a viscous syrup (0·075 g.), which crystallized on trituration with alcohol. 2 : 3 : 4 : 5-*Tetrabenzoyl* 1 : 6-*diphenyl mannitol* recrystallized from alcohol formed clusters of needles, m. p. 122—123° (Found : C, 74·0; H, 5·3. $C_{46}H_{38}O_{10}$ requires C, 73·6; H, 5·1%).

Condensation of 2 : 3-4 : 5-*Diiso*propylidene 1 : 6-*Dichloro*-1 : 6-*dideoxymannitol with Sodium Phenoxide.*—Diisopropylidene 1 : 6-dichloro-1 : 6-dideoxymannitol (0·5 g.) {the liquid isomer, n_D^{20} 1·4750, $[\alpha]_D$ −5·4° (in chloroform); see Wiggins, *J.*, 1946, 384} and sodium phenoxide (0·6 g.) in dry acetone (15 c.c.) were heated at 120° for 22 hours. The mixture was filtered hot and diluted with alcohol and water; the precipitated solid (0·4 g.) was collected and recrystallized from light petroleum (b. p. 100—120°), forming needles, m. p. 113—115·5° (Found : C, 67·5; H, 7·1. Calc. for $C_{21}H_{36}O_6$: C, 67·5; H, 6·9%). The product was 3 : 4-*iso*propylidene 1 : 6-diphenyl mannitol; the m. p. was not depressed on admixture with a specimen obtained from 3 : 4-*iso*propylidene 1 : 2-5 : 6-dianhydromannitol as described above. Hydrolysis with sulphuric acid and subsequent benzoylation of the product gave 1 : 6-diphenyl mannitol and its tetrabenzoate, respectively, the m. p. of which were unchanged on admixture with specimens prepared by the alternative method.

Treatment of 1 : 6-*Diphenyl* 3 : 4-iso*Propylidene Mannitol with Toluene*-p-*sulphonyl Chloride.*—(i) 1 : 6-Diphenyl 3 : 4-*iso*propylidene mannitol (0·5 g.) and toluene-*p*-sulphonyl chloride (1·0 g.) in dry pyridine (10 c.c.) were kept at 30° overnight and then poured into water. The precipitated solid recrystallized from alcohol as needles, m. p. 112—114°, alone or on admixture with the starting material. The recovery was almost quantitative. Repetition of the reaction at 60° for 5 hours also yielded only the starting material.

(ii) 1 : 6-Diphenyl 3 : 4-*iso*propylidene mannitol (0·5 g.) and toluene-*p*-sulphonyl chloride (1·0 g.) in dry pyridine (10 c.c.) were heated (oil-bath) at 120—130° for 8 hours, then poured into water and extracted with chloroform; the extract was washed successively with dilute sulphuric acid, sodium hydrogen carbonate solution, and water, and dried ($MgSO_4$). Evaporation of the solvent gave a syrup (0·7 g.), which crystallized on trituration with alcohol. The solid was recrystallized from light petroleum (b. p. 100—120°), yielding 1 : 6-*diphenyl* 3 : 4-iso*propylidene* 2 : 5-*ditoluene*-p-*sulphonyl mannitol* as needles, m. p. 107—108° (Found : C, 61·8; H, 5·6. $C_{35}H_{36}O_{10}S_2$ requires C, 61·6; H, 5·6%).

This ditoluene-*p*-sulphonate (0·10 g.) did not react with dry sodium iodide (0·25 g.) in dry acetone (10 c.c.) at 100° during 7½ hours. The starting material was recovered in 90% yield.

Hydrolysis. (i) The *iso*propylidene compound (0·26 g.) was heated under reflux with 0·1N-sulphuric acid (20 c.c.) and alcohol (25 c.c.) for 1½ hours. The solution yielded only unchanged starting material.

(ii) The *iso*propylidene compound (0·19 g.) was heated under reflux with 0·5N-sulphuric acid (20 c.c.) and alcohol (20 c.c.) for 8½ hours. The solution was then neutralized with sodium hydrogen carbonate and extracted with chloroform. Evaporation of the dried ($MgSO_4$) extract gave a crystalline residue (0·2 g.) which, recrystallized from alcohol–chloroform, afforded 1 : 6-*diphenyl* 2 : 5-*ditoluene*-p-*sulphonyl mannitol* in fine needles (0·1 g.), m. p. 185—186° (Found : C, 60·1; H, 5·6. $C_{32}H_{34}O_{10}S_2$ requires C, 59·8; H, 5·3%). This did not react with dry sodium iodide and dry acetone at 100° (8 hours); a small amount of sodium toluene-*p*-sulphonate was obtained on further heating at 150° for 8 hours.

Action of Phenol on Propylene Oxide.—To a solution of sodium (0·2 g.) and phenol (10 g.) in dry benzene (40 c.c.), propylene oxide (3 g.) was added and the mixture heated under reflux on the water-bath for 5 hours. After the mixture had cooled, water was added and the benzene layer separated; the aqueous layer was extracted with benzene, and the combined solutions were washed with dilute sodium hydroxide solution and with water and finally dried ($MgSO_4$). Evaporation of the benzene gave a residual oil (2·8 g.) which was distilled under reduced pressure; 1-phenoxypropan-2-ol was collected as a colourless oil (1·9 g.), b. p. (bath-temp.) 134—136°/15 mm., n_D^{17} 1·5239. Hurd and Perlitz (*loc. cit.*) record b. p. 130°/21 mm., n_D 1·5232.

1-*Phenoxyprop*-2-*yl Toluene*-p-*sulphonate*.—1-Phenoxypropan-2-ol (0·57 g.) and toluene-*p*-sulphonyl chloride (1·1 g.) in dry pyridine (5 c.c.), were kept, first at 0° and then at about 30° (overnight), the solution was poured into water, and the solid derivative (1·2 g.) collected, washed with water, and recrystallized from aqueous alcohol, giving 1-*phenoxyprop*-2-*yl toluene*-p-*sulphonate* in clusters of fine needles (0·9 g.), m. p. 93—94° (Found: C, 62·5; H, 5·9. $C_{16}H_{18}O_4S$ requires C, 62·8; H, 5·9%).

The toluene-*p*-sulphonyl derivative (0·151 g.) was heated with dry sodium iodide (0·36 g.) and dry acetone (10 c.c.) at 95—100° for 6 hours. The precipitate of sodium toluene-*p*-sulphonate was then collected, washed with a little dry acetone, and dried at 110°; the yield was 0·088 g. (92%).

Phenoxyacetaldehyde.—Phenoxyacetaldehyde diethylacetal (Dey, *J.*, 1937, 1057) (5 g.) was hydrolysed on the boiling water-bath for 1 hour with 1% sulphuric acid (50 c.c.) and acetic acid (20 c.c.). The reaction mixture was neutralised with sodium hydrogen carbonate and extracted with ether. The extract was dried ($MgSO_4$) and evaporated to give an oil (3·9 g.) which, on distillation, yielded phenoxyacetaldehyde (1·9 g.), b. p. (bath-temp.) 130—135°/15 mm., n_D^{20} 1·5321. Rotbart (*Ann. Chim.*, 1934, **1**, 479), records b. p. 101—103°/15 mm., n_D^{21} 1·5381. The product gave a semicarbazone, m. p. 145°, in agreement with the value recorded by Rotbart.

Action of Methylmagnesium Iodide on Phenoxyacetaldehyde.—Phenoxyacetaldehyde (0·93 g.) in dry ether (7 c.c.) was added dropwise at room temperature to methylmagnesium iodide (from Mg, 0·2 g.) in ether (10 c.c.). The mixture was heated under reflux on the water-bath for 1½ hours and thereafter poured into ice-water acidified with sulphuric acid. The ethereal layer was separated, the aqueous layer was extracted with more ether, and the combined ether extracts were washed successively with sodium hydrogen carbonate solution and water. Evaporation of the dried ($MgSO_4$) solution gave a residual oil (1·0 g.) from which 1-phenoxypropan-2-ol (0·49 g.) distilled at 138—140° (bath-temp.)/15 mm. It showed n_D^{20} 1·5269. Treatment of the product (0·15 g.) with toluene-*p*-sulphonyl chloride in pyridine as described above gave 1-phenoxyprop-2-yl toluene-*p*-sulphonate (0·20 g.), m. p. 91—92·5° alone or in admixture with a specimen of the compound obtained from propylene oxide.

Action of p-*Chlorophenol on* **3**: 4-iso*Propylidene* **1**: **2**-**5**: **6**-*Dianhydromannitol*.—Sodium (0·25 g.) was dissolved, with warming, in a solution of *p*-chlorophenol (1·4 g.) in dry benzene (70 c.c.) and *iso*propylidene dianhydromannitol (0·5 g.) added. After the mixture had been heated under reflux for 8 hours, it was cooled, decanted from a little unchanged sodium, and shaken with 20% sodium hydroxide solution. The benzene layer was separated, washed with water, and dried ($MgSO_4$). Evaporation of the solvent gave a residual syrup which crystallised on trituration with chloroform–light petroleum (b. p. 40—60°); recrystallization from the same mixture of solvents yielded **1**: **6**-*di*-p-*chlorophenyl* **3**: 4-iso*propylidene mannitol* as needles (0·3 g.), m. p. 111—114° (Found: C, 57·0; H, 5·6. $C_{21}H_{24}O_6Cl_2$ requires C, 57·1; H, 5·4%).

The *iso*propylidene compound (0·1 g.) was heated under reflux with N-sulphuric acid (25 c.c.) and alcohol (30 c.c.), for 10 hours. The solid which separated on cooling was collected, washed with water, and recrystallized from aqueous alcohol, forming **1**: **6**-*di*-p-*chlorophenyl mannitol* as colourless needles (0·06 g.), m. p. 177—178° (Found: C, 53·6; H, 4·8. $C_{18}H_{20}O_6Cl_2$ requires C, 53·6; H, 5·0%).

Action of m-*Cresol on* **3**: 4-iso*Propylidene* **1**: **2**-**5**: **6**-*Dianhydromannitol*.—*m*-Cresol (4·06 g.) was dissolved in dry benzene (50 c.c.) and sodium (0·25 g.) added in small pieces. After the reaction had ceased, *iso*propylidene dianhydromannitol (1·0 g.) was added and the reaction carried out as in the case of the *p*-chlorophenol. The syrup obtained on evaporation of the benzene solution distilled as a yellow viscid syrup at 160—170° (bath-temp.)/0·05 mm. (Found: C, 68·1; H, 7·7. Calc. for $C_{23}H_{30}O_6$: C, 68·7; H, 7·5%). The syrup crystallized on trituration with methyl alcohol and recrystallized from methyl alcohol–water as colourless needles of **3**: 4-iso*propylidene* **1**: **6**-*di*-m-*tolyl mannitol monohydrate* (0·9 g.), m. p. 68—70°, $[\alpha]_D^{17}$ +22·4° (*c*, 3·22 in pyridine) (Found: C, 68·6; H, 7·7. $C_{23}H_{30}O_6,H_2O$ requires C, 68·7; H, 7·6%). The substance becomes liquid when dried over phosphoric anhydride.

This compound (0·73 g.) was heated under reflux for 7 hours with 0·1N-sulphuric acid (30 c.c.). After the solution had been allowed to cool, the solid formed was collected by filtration and recrystallized from absolute alcohol, to yield **1**: **6**-*di*-m-*tolyl mannitol* (0·31 g.) as small plates, m. p. 139·5—140·5°, $[\alpha]_D^{15}$ +24·6° (*c*, 1·38 in pyridine) (Found: C, 66·5; H, 7·2. $C_{20}H_{26}O_6$ requires C, 66·3; H, 7·2%). The di-*m*-tolyl mannitol (0·08 g.) was dissolved in dry pyridine, treated with benzoyl chloride (0·3 g.) at 0°, and kept at 30° overnight. The usual treatment afforded a syrup which crystallized on trituration with methyl alcohol. Recrystallization from the same solvent yielded **2**: **3**: **4**: **5**-*tetrabenzoyl* **1**: **6**-*di*-m-*tolyl mannitol* as

colourless needles, m. p. 101—102°, $[\alpha]_D$ +30·2° (*c*, 2·98 in pyridine) (Found : C, 73·9; H, 5·5. $C_{48}H_{42}O_{10}$ requires C, 74·0; H, 5·4%).

Action of o-*Cresol on* 1 : 3-2 : 4-*Diethylidene* 5 : 6-*Anhydrosorbitol.*—*o*-Cresol (14·4 g.) was dissolved in dry benzene (10 c.c.), and sodium (0·9 g.) added in small pieces. When the reaction had ceased, diethylidene anhydrosorbitol (7·2 g.) was added and the mixture heated under reflux for 9 hours. It was then diluted with benzene, water added, and the benzene layer separated and washed with sodium hydroxide solution. After being washed with water, the extract was dried ($MgSO_4$) and evaporated to a syrup which was dissolved in hot aqueous alcohol. On cooling, the solution yielded 1 : 3-2 : 4-*diethylidene* 6-o-*tolyl sorbitol* (7·0 g.) as prisms, m. p. 134·5—135·5°, $[\alpha]_D^{18}$ +4·07° (*c*, 4·94 in chloroform) (Found : C, 62·9; H, 7·5. $C_{17}H_{24}O_6$ requires C, 63·0; H, 7·4%).

This diethylidene compound (0·5 g.) and toluene-*p*-sulphonyl chloride (0·5 g.) in dry pyridine (8 c.c.) at 25° (3 days) gave 1 : 3-2 : 4-*diethylidene* 5-*toluene*-p-*sulphonyl* 6-o-*tolyl sorbitol* (0·41 g.) as colourless needles, m. p. 143—144°, $[\alpha]_D^{20·5}$ −14·8° (*c*, 2·55 in dry pyridine), from aqueous alcohol (Found : C, 60·0; H, 6·1; S, 7·0. $C_{24}H_{30}O_8S$ requires C, 60·3; H, 6·3; S, 6·7%); this did not react with dry sodium iodide in dry acetone at 105—110° (7 hours), the starting material being recovered in 83% yield.

Hydrolysis. Diethylidene *o*-tolyl sorbitol (6·0 g.) was heated under reflux with 2N-sulphuric acid (20 c.c.) and alcohol (20 c.c.) for 6 hours. The solution was neutralised with barium carbonate and filtered. Evaporation of the filtrate yielded liquid 6-o-*tolyl sorbitol.* After being dried at 50°/0·01 mm. it showed $[\alpha]_D$ +12·4° (*c*, 7·7 in ethyl alcohol) (Found : C, 57·4; H, 7·4. $C_{13}H_{20}O_6$ requires C, 57·4; H, 7·4%). It formed a crystalline *penta-acetate*, m. p. 89·5—90·5° (Found : C, 57·2; H, 6·2. $C_{23}H_{30}O_{11}$ requires C, 57·3; H, 6·2%).

Action of m-*Cresol on* 1 : 3-2 : 4-*Diethylidene* 5 : 6-*Anhydrosorbitol.*—*m*-Cresol (3 g.) was dissolved in dry benzene (35 c.c.) and treated with sodium (0·9 g.) and diethylidene anhydrosorbitol (1·3 g.) as in the case of the *o*-isomer. The syrupy 1 : 3-2 : 4-*diethylidene* 6-m-*tolyl sorbitol* obtained on evaporation of the benzene solution crystallized in the form of colourless plates (1·4 g.), m. p. 88°, $[\alpha]_D^{19}$ −8·1° (*c*, 3·46 in pyridine) (Found : C, 62·9; H, 7·8%).

The *m*-tolyl ether was hydrolysed as described for the *o*-isomer. The 6-m-*tolyl sorbitol* obtained was dried at 50°/0·005 mm. and showed $[\alpha]_D^{18·5}$ +13·9° (*c*, 6·06 in ethyl alcohol) (Found : C, 57·2; H, 7·7%). Acetylation with acetic anhydride and anhydrous sodium acetate afforded stout prisms of the *penta-acetate*, m. p. 110—111°, $[\alpha]_D^{18}$ −28·5° (*c*, 3·72 in pyridine) (Found : C, 57·0; H, 6·4%).

Action of Phenol on 1 : 3-2 : 4-*Diethylidene* 5 : 6-*Anhydrosorbitol.*—Phenol (1·3 g.) was dissolved in dry benzene (30 c.c.), sodium (0·32 g.) added, followed by diethylidene anhydrosorbitol (1 g.), and the mixture was treated as above. The syrup obtained on evaporation of the benzene solution crystallized from light petroleum (b. p. 40—60°) as colourless needles of 1 : 3-2 : 4-diethylidene 6-phenyl sorbitol (0·7 g.), m. p. 98—99°, $[\alpha]_D^{17·5}$ +11·3° (*c*, 2·82 in ethyl alcohol).

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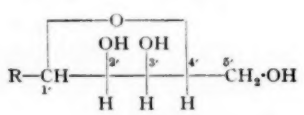
12. *Nucleotides. Part IX.* The Synthesis of Adenylic Acids* a *and* b *from 5′-Trityl Adenosine.*

By D. M. Brown and A. R. Todd.

Phosphorylation of 5′-trityl adenosine with dibenzyl chlorophosphonate, followed by removal of protecting groups, yields two adenylic acids, identical in all respects with the isomeric adenylic acids *a* and *b* derived from ribonucleic acids, and evidence is presented for their formulation as adenosine-2′ phosphate and adenosine-3′ phosphate, although not necessarily respectively. Their interconversion under acidic conditions is explained by facile phosphoryl migration, a concept which clarifies some other properties of the acids and their derivatives.

Earlier work in these and other laboratories has been directed to the final structural elucidation and total synthesis of the natural ribonucleosides adenosine, guanosine, uridine, and cytidine obtained by hydrolysis of ribonucleic acids. They can now be represented by the general formula (I) in which R represents the purine (adenine or guanine) or pyrimidine (uracil or cytosine) residue, D-ribose being linked in the β-orientation to $N_{(9)}$ in the case of the purine and to $N_{(3)}$ in the case of the pyrimidine nucleosides. In the present series of papers, attention has been directed to, *inter alia*, the synthesis of their monophosphates, the ribonucleotides, using, if possible, methods which would define without ambiguity the location of the phosphoryl group in the synthetic products. To date, unambiguous syntheses have been recorded for adenosine-5′ phosphate (muscle adenylic acid) (Baddiley and Todd, *J.*, 1947, **648**; Levene and Tipson, *J. Biol. Chem.*, 1937, **121**, 131; Bredereck, Berger, and Ehrenberg, *Ber.*, 1940, **73**, 269), for uridine-5′ phosphate (Levene and Tipson, *J. Biol. Chem.*, 1934, **106**, 113; Michelson and Todd, *J.*, 1949, 2476), and for guanosine-5′ phosphate and cytidine-5′ phosphate (Michelson and Todd, *loc. cit.*).

For many years it was generally accepted that only four mononucleotides could be isolated from chemical hydrolysates of ribonucleic acids. The location of the phosphoryl group in these substances was studied by Levene and Harris (*J. Biol. Chem.*, 1932, **98**, 9; 1933, **101**, 419) who, on the basis of certain degradative experiments on yeast adenylic and guanylic acids, concluded that these nucleotides were the 3′-phosphates of the corresponding nucleosides. The stability of the glycosidic linkage in the pyrimidine nucleotides rendered similar degradative procedures impracticable but it was assumed by analogy that they too were 3′-phosphates. Michelson and Todd (*loc. cit.*), using a synthetic route which, on available evidence, seemed unambiguous, synthesised nucleoside-3′ phosphates and did in fact obtain adenylic and uridylic acids which were identical with the natural products and gave identical derivatives. Their synthetic route involved as



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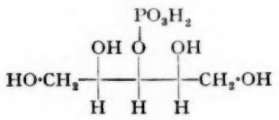


(II)

key intermediates the benzylidene nucleosides whose formulation as 3′ : 5′-benzylidene derivatives appeared secure from the degradative work on benzylidene guanosine carried out by Gulland and Overend (*J.*, 1948, 1380; cf. also Bredereck and Berger, *Ber.*, 1940, **73**, 1124). The same benzylidene derivatives were used to prepare the so-called 2′-phosphates of the nucleosides by direct phosphorylation and removal of the benzylidene group (Gulland and Smith, *J.*, 1947, 338; 1948, 1527; Michelson and Todd, *loc. cit.*).

* Part VIII, *J.*, 1951, 2952.

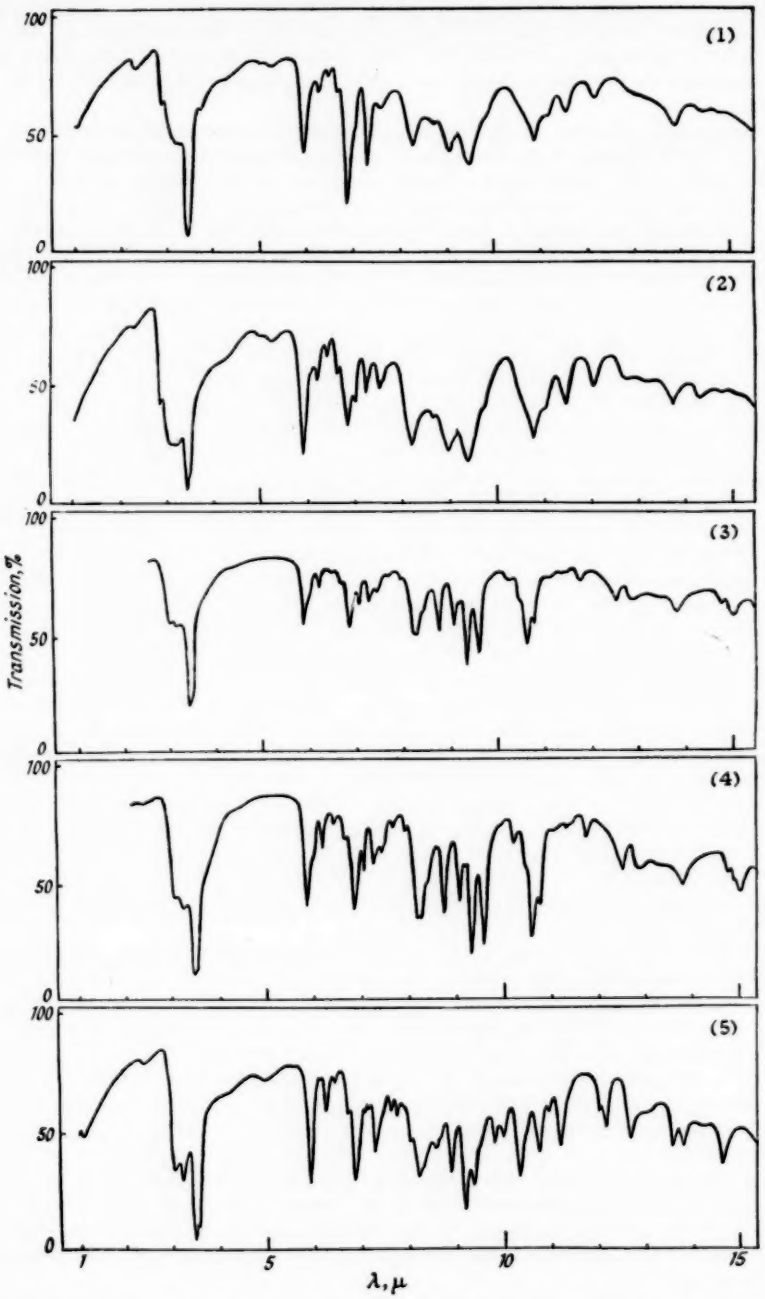
Subsequent work (Brown, Haynes, and Todd, *J.*, 1950, 2299) showed that the benzylidene nucleosides used were in fact 2′ : 3′-benzylidene derivatives; it followed that the "2′-phosphates" were in fact 5′-phosphates and that the syntheses of the natural nucleotides by Michelson and Todd (*loc. cit.*), although they gave the natural products, were ambiguous in that they proved only that the phosphoryl group was either at $C_{(2')}$ or at $C_{(3')}$.

An important advance in nucleic acid chemistry was made by Carter and Cohn (*Fed. Proc.*, 1949, **8**, 190) when they succeeded in separating from alkaline hydrolysates of yeast ribonucleic acid two isomeric adenylic acids which they termed *a* and *b*. This was followed by the separation into two isomers of crude guanylic, uridylic, and cytidylic acids in similar hydrolysates (Cohn, *J. Amer. Chem. Soc.*, 1950, **72**, 1471, 2811; Loring, Luthy, Bortner, and Levy, *ibid.*, p. 1471). Carter and Cohn (*loc. cit.*; see also Carter, *J. Amer. Chem. Soc.*, 1950, **72**, 1466) degraded the isomeric adenylic acids *a* and *b* to adenine and adenosine and suggested that they were adenosine-2′ and adenosine-3′ phosphates, adenosine-5′ phosphate being excluded as a possible structure for either on enzymic evidence and on chemical grounds, including their stability to periodic acid (Brown, Haynes, and Todd, *loc. cit.*). Other types of isomerism, however, were not excluded. Their view that adenylic acid *b* was adenosine-3′ phosphate rested on its identity with the previously known and synthetically available yeast adenylic acid; as has already been mentioned, however, the synthetic route to this material did not in fact prove the location of the phosphoryl group. It seemed that a solution of the constitutional problem might be most directly obtained by unambiguous synthesis of either or both of the isomeric nucleotides. It may be stated at once that this objective has not been reached. With the demonstration that the condensation product of adenosine with benzaldehyde is 2′ : 3′-benzylidene adenosine exactly analogous to the condensation product with acetone, the problem of differentiating the $C_{(2')}$ and the $C_{(3')}$ positions became much more difficult. Many examples are known in carbohydrate chemistry in which benzaldehyde condenses with 1 : 3-glycol systems to produce cyclic benzylidene compounds (cf. Haworth and Hirst, *Ann. Rev. Biochem.*, 1936, **5**, 82), and its failure to do so in the case of the nucleosides made it probable that no better results would be obtained with other reagents. Moreover, in alkylation and acylation no preferential reaction at $C_{(2')}$ or $C_{(3')}$ has been observed. Nevertheless, many experiments were carried out with a variety of reagents in the hope of preparing a 3′ : 5′-substituted adenosine; since no success attended them they are not described here. It is apparent from a consideration of the stereochemistry of the ribofuranose residue present in the nucleosides that the $C_{(2')}$ and the $C_{(3')}$ positions are very similar and that the relation between the hydroxyl groups at $C_{(3')}$ and $C_{(5')}$ is not favourable to the formation of 3′ : 5′-cyclic derivatives; further evidence pointing in the same direction has come from a study of the phosphoryl migration in adenylic acid derivatives discussed in some detail in the following paper.

Attention was therefore directed to the products obtained from the phosphorylation of 5′-substituted nucleosides, since Brown, Haynes, and Todd (*loc. cit.*) had already noticed that on phosphorylating $N_{(6)}$: 5′-dityrityl adenosine and removing protecting groups a product was obtained which on paper chromatography showed two spots corresponding to adenylic acids *a* and *b*. Treatment of 5′-trityl adenosine (Levene and Tipson, *J. Biol. Chem.*, 1937, **121**, 131) with dibenzyl chlorophosphonate in the usual manner gave a product having the composition of 5′-trityl adenosine dibenzyl phosphate in excellent yield. This was hydrogenated to remove benzyl groups, then converted into its lead salt, and the latter suspended in water and decomposed with hydrogen sulphide, a process which simultaneously removed the 5′-trityl group (cf. Michelson and Todd, *loc. cit.*). From the filtered solution obtained, free nucleotides could be isolated but the yield was always low. The solution was, however, shown to contain only two components, corresponding to adenylic acids *a* and *b* which could be separated in crystalline form after ion-exchange chromatography.

The low yields obtained above made this isolation procedure unattractive and for large-scale preparation the following method was adopted. The 5′-trityl adenosine dibenzyl phosphate was treated with 80% acetic acid, causing removal of the trityl group

Spectra of solids in Nujol.



1, ***Synthetic adenylic acid*** a. 2, *Natural adenylic acid* a. 3, *Synthetic adenylic acid* b. 4, *Natural adenylic acid* b. 5, *Adenosine-5′ phosphate.*

and partial debenzylation. Hydrogenation then gave the mixed adenylic acids, isolated as a barium salt in an overall yield of some 50%. On the evidence of paper chromatography, this material contained no nucleotides other than adenylic acids *a* and *b*. Application of anion-exchange chromatography by the method used by Cohn (*loc. cit.*) for the separation of the natural acids separated the synthetic mixture into two crystalline isomeric adenylic acids. These were rigidly identified as adenylic acid *a* and adenylic acid *b* by comparison with specimens of the natural acids (kindly supplied by Drs. Carter, Cohn, and Doherty) by comparing their ion-exchange and paper-chromatographic behaviour, *X*-ray powder photographs, and infra-red absorption spectra (figure). The synthetic adenylic acid *b* was also obtained as a beautifully crystalline hydrate which gave a different *X*-ray powder photograph but was otherwise similar to the anhydrous acid. In view of the complexity of the *X*-ray diffraction photographs we have not indexed the lines and have relied on visual comparison. Clark (*Arch. Biochem. Biophys.*, 1951, **31**, 18) has recorded data from *X*-ray powder photographs of yeast adenylic acid without, however, indicating which isomer was examined. Blout and Fields (*J. Biol. Chem.*, 1949, **178**, 335) have also published an infra-red spectrum of yeast adenylic acid; our spectra (figure) show considerably greater resolution, and for completeness we have included also the spectrum of adenosine-5′ phosphate (muscle adenylic acid).

Having established that the phosphorylation of 5′-trityl adenosine yields the isomeric adenylic acids *a* and *b*, it is necessary to enquire whether these acids are indeed the 2′- and 3′-phosphates of adenosine. It is clear from the stability of both acids to periodate that they contain no free α-glycol system. Again, it may be argued that in general monoesterification of a molecule containing two similar hydroxyl groups would be expected to yield isomers differing in the location of the acyl group. In the present instance, the production of only two isomeric acids is strong presumptive evidence for their formulation as the 2′- and 3′-phosphates. Carter (*loc. cit.*) suggested the alternative possibility that both acids might be 3′-phosphates and that they might differ by virtue of α-β-isomerism at the glycosidic linkage. Doherty (Abstr. 118th Meeting Amer. Chem. Soc., 1950, 56c) has carried out degradative experiments which at first sight appear to favour this view. The original work of Levene and Harris (*locc. cit.*) on yeast adenylic acid rested on removal of the sugar phosphate residue from a nucleotide under acid conditions followed by its reduction to a ribitol phosphate; since their product was optically inactive, they formulated it as ribitol 3-phosphate (II) and hence drew the conclusion that the phosphoryl group in adenylic acid was at position 3′. Doherty (*loc. cit.*) treated both the adenylic acids *a* and *b* with dry hydrogen chloride in benzyl alcohol and then hydrogenated the resulting benzyl riboside phosphate to a ribitol phosphate. In each case he obtained optically inactive products and this he held to indicate that they were derived from riboside-3′ phosphates, *i.e.*, that *a* and *b* were α- and β-adenosine-3′ phosphates. There are, however, a number of objections to this conclusion. Only one adenosine has been obtained from natural sources, including ribonucleic acids, and this has been shown to have the β-configuration (Davoll, Lythgoe, and Todd, *J.*, 1946, 833; Clark, Todd, and Zussman, *J.*, 1951, 2952); no example of α-β-isomerism among any of the natural nucleosides has ever been observed. Moreover, we observed that adenylic acids *a* and *b* undergo ready interconversion in dilute acidic solutions to an equilibrium mixture containing approximately equal amounts of the two isomers. A similar interconversion occurs in the case of the isomeric pyrimidine nucleotides from ribonucleic acid (Cohn, *J. Amer. Chem. Soc.*, 1950, **72**, 2811). This interconversion is inherently unlikely to result from an α-β-isomerisation since in that case one form, being more stable, would be expected to predominate (Howard, Kenner, Lythgoe, and Todd, *J.*, 1946, 861) and isomerisation would be accompanied by furanose–pyranose changes. Since such α-β-interconversions depend on the electron availability at the nitrogen atom attached to $C_{(1)}$ of the sugar residue (Howard, Kenner, Lythgoe, and Todd, *J.*, 1946, 855) it is very unlikely on theoretical grounds that they should occur in adenosine derivatives where $N_{(9)}$ in the purine residue has a very low basicity; this argument also applies to other natural and synthetic nucleosides and their derivatives.

All the properties of adenylic acids *a* and *b* (and equally those of the other isomeric

pairs) are, however, easily explained on the basis that they are 2′- and 3′-phosphates and that their interconversion is due to facile phosphoryl migration. That a migration of this type is to be expected in acid solution is clear from the analogous acid-catalysed phosphoryl migration in the monophosphates of glycerol (Bailly, *Compt. rend.*, 1938, **206**, 1902; 1939, **208**, 443, 1820; Verkade, Stoppelenburg, and Cohen, *Rec. Trav. chim.*, 1940, **59**, 886). Verkade *et al.* (*loc. cit.*) have also shown that the glycerol monophosphates are stable without rearrangement in alkaline solution, an observation which we have also made with adenylic acids *a* and *b*. This phosphoryl migration suggests a probable explanation for the lack of optical activity in the ribitol phosphates obtained by Levene and Harris (*locc. cit.*) and by Doherty (*loc. cit.*) in their degradations of adenylic acids, since if at any time the phosphoryl group becomes attached to the symmetrical $C_{(3)}$ position (cf. II) further migration will lead to the production of racemates. On this view, the evidence obtained from such degradative experiments is unreliable and the conclusion must be drawn that in no case has the position of the phosphoryl residue in any of the natural nucleotides (other than the 5′-nucleotides) been established. In our view, the weight of evidence indicates that the isomeric adenylic acids *a* and *b* are the 2′- and 3′-phosphates of adenosine, although which is the 2′- and which the 3′- remains to be determined. The other pairs of isomeric nucleotides have almost certainly analogous structures.

In the course of the synthesis of adenylic acids *a* and *b* described above, the initially prepared 5′-trityl adenosine dibenzyl phosphate was treated with 80% acetic acid, and the water-soluble product hydrogenated to yield the nucleotides. In one experiment hydrogenation was slow and incomplete, and after conversion of the products into barium salts there was obtained, in addition to the barium salt of adenylic acids *a* and *b*, a crude barium salt of what was evidently a mixture of the monobenzyl esters of these acids. By paper-chromatographic methods it was found that acid hydrolysis of this material yielded adenine, whereas hydrogenation converted it into a mixture of adenylic acids *a* and *b*. Moreover, by treating the mixed adenylic acids *a* and *b* with phenyldiazomethane in dimethylformamide and neutralisation of the product with barium hydroxide, it was possible to isolate a barium salt having the analytical composition of a monobenzyl ester of adenylic acid and showing the same paper-chromatographic behaviour as that of the material obtained above. Two components were present in the barium salt prepared by either route, corresponding to the *a* and *b* isomers, and it was possible to relate them to their corresponding adenylic acid. We have not isolated the separate isomeric esters, since they appear to undergo interconversion reactions very readily during the working up of the phenyldiazomethane reaction product. In the phenyldiazomethane reaction, small amounts of the dibenzyl esters are also produced but we have not studied them further.

The monobenzyl esters of adenylic acids *a* and *b* obtained above are very readily and completely debenzylated by both acids and alkali, with simultaneous phosphoryl migration (*i.e.*, production of a mixture of the two nucleotides). This interesting behaviour in which diesters of phosphoric acid show a remarkable instability towards both acid and alkali has a counterpart in the behaviour of the esters of glycerol monophosphates (Bailly and Gaumé, *Bull. Soc. chim.*, 1935, **2**, 354; Baer and Kates, *J. Biol. Chem.*, 1948, **175**, 79). Its mechanism will be discussed more fully elsewhere (Brown and Todd, following paper), together with its important implications with regard to the hydrolytic behaviour and structure of the nucleic acids.

Experimental

M. p.s are uncorrected. *X*-Ray powder photographs were taken by using a Metropolitan-Vickers Raymax crystallographic unit and a Unicam standard powder camera (19-cm. diam.; Cu-K_α radiation). The infra-red absorption spectra were determined by using a Perkin–Elmer Model 21, self-recording, double-beam instrument with sodium chloride prisms.

Paper Chromatography of Some Adenine Derivatives.—For the paper-chromatographic analysis of the products of the reactions described here two solvent systems were used. The 5% disodium hydrogen phosphate–*iso*amyl alcohol (hereafter, sodium phosphate) solvent of Carter (*loc. cit.*) gave readily reproducible R_F values within small limits. The values quoted in the

annexed Table are slightly lower than those given by Carter for those substances which he studied. In the butanol–acetic acid–water (butanol–acetic acid) solvent system (Partridge, *Biochem. J.*, 1948, **42**, 238) R_F values varied widely in different chromatograms, particularly when run downwards; those values quoted were typical (not average) for upward-run chromatograms. This solvent had the advantage of separating the adenylic acids widely from their benzyl esters (which were well resolved) and from non-phosphorylated derivatives. In chromatograms using either solvent standard compounds were always run simultaneously. Adenylic acid *a* is not distinguished from adenosine-5′ phosphate in the sodium phosphate solvent (Carter, *loc. cit.*), nor are the three adenylic acids characteristically resolved by butanol–acetic acid. For the detection of spots, chromatograms were photographed on Ilford Reflex Document Paper No. 50 in ultra-violet light (Markham and Smith, *Biochem. J.*, 1949, **45**, 294).

Substance	R_F values: 5% Na_2HPO_4–*iso*amyl alcohol	R_F values: Butanol–acetic acid–water (4 : 1 : 5)
Adenine	0·36	0·57
Adenosine	0·50	0·48
Adenylic acid *a*	0·69	0·15
Adenylic acid *b*	0·62	—
Adenosine-5′ phosphate	—	0·15
Adenosine benzyl hydrogen phosphate *a*	0·67	0·44
Adenosine benzyl hydrogen phosphate *b*	0·56	0·50
Adenosine-5′ benzyl hydrogen phosphate	0·65	0·41

5′-Trityl Adenosine Dibenzyl Phosphate.—A solution of 5′-trityl adenosine (3·1 g.) in dry pyridine (60 c.c.) at a temperature slightly above the m. p. of the solution was treated with dibenzyl chlorophosphonate (from 4·5 g. of dibenzyl hydrogen phosphite) with exclusion of moisture. The solution was kept at this temperature, with occasional shaking, for 4 hours and then left at room temperature overnight. A solution of sodium carbonate (3·0 g.) in water (15 c.c.) was added, sodium chloride removed by filtration, and the filtrate evaporated to dryness under reduced pressure. The residue was evaporated under reduced pressure several times with ethanol to remove pyridine completely. The residual pale brown gum was shaken with chloroform and sodium hydrogen carbonate solution, and the chloroform layer further washed with sodium hydrogen carbonate and then water and dried (Na_2SO_4). After evaporation to dryness, the residue was dissolved in ethanol (15 c.c.) and set aside for 48 hours, whereupon unchanged 5′-trityl adenosine (0·24 g.) separated. After filtration, the solution was evaporated to dryness and the resulting solid foam refluxed with dry ether (25 c.c.) for 2 hours. The granular *phosphate* (4·7 g.) was hygroscopic but free-flowing when dry (Found, in material dried at 55° *in vacuo* over phosphoric oxide: C, 65·6; H, 5·5; N, 8·8. $C_{43}H_{40}O_7N_5P$ requires C, 67·2; H, 5·2; N, 9·1%. $C_{43}H_{40}O_7N_5P,H_2O$ requires C, 65·6; H, 5·3; N, 8·9%).

Adenylic Acids a *and* b *from 5′-Trityl Adenosine Dibenzyl Phosphate.*—(a) *Lead salt method.* The above dibenzyl ester (1·0 g.) was dissolved in ethanol (50 c.c.) and water (5 c.c.) and hydrogenated at room temperature and pressure over palladised charcoal (70 mg.) and palladium oxide (30 mg.). Hydrogenation was slow (85 c.c. of hydrogen absorbed in 24 hours). After removal of catalyst, the solution was evaporated to dryness under reduced pressure, and the residual glass was dissolved in chloroform and poured into light petroleum (b. p. 40—60°). The crude 5′-trityl adenosine phosphate was precipitated as a hygroscopic solid (0·72 g.) (Found: C, 60·9; H, 5·5; N, 9·0. Calc. for $C_{29}H_{28}O_7N_5P$: C, 59·2; H, 4·8; N, 11·7%). This material (0·6 g.) was converted into the lead salt (0·55 g.) by addition of lead acetate solution to its neutralised solution in aqueous ethanol (Found: C, 45·7; H, 3·9; N, 7·4. Calc. for $C_{29}H_{26}O_7N_5PPb$: C, 44·0; H, 3·3; N, 8·8%). The lead salt was suspended in water containing a little ethanol and saturated with hydrogen sulphide. Lead sulphide was filtered off and extracted with hot water, and the combined filtrate and extracts were evaporated to small bulk. After filtration from a little triphenylcarbinol, the mixed nucleotides (43 mg.) were precipitated by addition of four volumes of ethanol. Presence of only two substances, corresponding to adenylic acids *a* and *b*, in this product was disclosed on paper chromatograms. Extraction of the separated substances from the paper and quantitative determination by measurement of the optical density at 260 mμ showed that they were present in approximately equal amount. A portion (13 mg.) of the mixture was separated into two components by ion-exchange chromatography (Amberlite IRA-400 resin) on a small column, as described below. An elution diagram was obtained identical with that given by a mixture of natural

adenylic acids *a* and *b* on the same column. The products were isolated and crystallised from water. Paper-chromatographic analysis showed that complete separation had been achieved and that the two products were indistinguishable from adenylic acids *a* and *b*.

(b) *Preparative method.* A solution of the dibenzyl phosphate (0·41 g.) in 80% acetic acid (4 c.c.), was boiled under reflux for 20 minutes. On cooling, crystalline triphenylcarbinol separated. Water and chloroform were added and the aqueous phase was separated, washed with more chloroform, and evaporated. The product was freed from acetic acid by evaporation with ethanol under reduced pressure. The residual gum (0·22 g.) was dissolved in water and hydrogenated over palladium charcoal–palladium oxide (38 c.c. absorbed). Catalyst was filtered off and the solution evaporated. The product (0·187 g.) was converted into the barium salt which was purified by precipitation several times from its aqueous solution by addition of ethanol (3 vols.) and formed a white powder (0·126 g., 49%) (Found: C, 24·5; H, 3·1; N, 13·3. Calc. for $C_{10}H_{12}O_7N_5PBa$: C, 24·9; H, 2·5; N, 14·5%). A paper-chromatographic study of these reactions showed that the product obtained after the acetic acid hydrolysis contained traces of adenine and adenosine, adenylic acids *a* and *b* (which were in one case isolated), the monobenzyl esters of these acids (see below), and an unidentified component (R_P 0·83 in sodium phosphate and 0·70 in butanol–acetic acid). The last substance, which contained phosphorus, was not present in the solution after hydrogenation, only adenylic acids *a* and *b* and traces of adenosine and adenine being observed.

Separation of Adenylic Acids a *and* b *from the Mixture of Synthetic Barium Salts of the Acids.*—The barium salt (414 mg.) described above was dissolved in water (90 c.c.) and run slowly on to a column (15 cm. × 4 sq. cm.) of Dowex-2 anion-exchange resin (mesh size 200—400) in the formate form. The column was water-washed until barium ion was no longer present in the effluent (150 c.c.). Elution was continued with 0·096N-formic acid at a flow rate of 3 c.c./minute and the eluate collected in 25-c.c. fractions in an automatically operated fraction collector. The progress of elution of the products was followed by the optical density of the fractions at 260 and 280 mμ. After the first component (adenylic acid *a*) had been eluted (volume to peak, *ca.* 3·5 l.), the second component (adenylic acid *b*) was removed from the column with N-formic acid.

The fractions containing adenylic acid *a* were united and concentrated, below room temperature under reduced pressure, to small bulk (50 c.c.), and the product was isolated by freeze-drying this solution. It was recrystallised three times from water (charcoal), giving *adenylic acid* a as a colourless crystalline solid (67·2 mg.), m. p. 187° (decomp.) undepressed by a sample of natural adenylic acid *a* [m. p. 187° (decomp.)] (Found, in material dried at 115°/0·1 mm.: C, 34·9; H, 4·3; N, 19·8. $C_{10}H_{14}O_7N_5P$ requires C, 34·6; H, 4·1; N, 20·2%. Uptake of periodate: 0·0 mol./mol.). The *X*-ray powder photograph and infra-red spectrum (figure) of this material and of natural adenylic acid *a* were identical. The compounds were indistinguishable on paper chromatograms, the synthetic compound travelling as one component.

The fractions containing adenylic acid *b* were set aside at 0° overnight. From the peak fraction *adenylic acid* b separated in long, fine colourless needles (56·3 mg.), m. p. 197° (decomp.) and mixed m. p. 195° (decomp.) with natural adenylic acid *b* [m. p. 193° (decomp.)] (Found, in air-dried material: C, 34·1; H, 4·3; N, 20·5%). The *X*-ray powder photograph and infra-red spectrum of this compound were identical in all respects with those of a recrystallised sample of natural adenylic acid *b*. By freeze-drying these fractions a further quantity of adenylic acid *b* was obtained which crystallised from water as a *hydrate* in prismatic needles (63·7 mg.) (Found, in material dried at room temp.: C, 31·9; H, 4·5; N, 18·5. $C_{10}H_{14}O_7N_5P,1{\cdot}5H_2O$ requires C, 31·9; H, 4·5; N, 18·7%. Found, in material dried at 110°: C, 35·0; H, 4·1; N, 20·0%. Periodate uptake: 0·0 mol./mol.). The hydrate was identical with the natural material on paper chromatograms but gave a different *X*-ray powder diagram. The infra-red spectrum was, however, the same as that of the uncrystallised natural compound in the position of the absorption bands, which differed only slightly in relative intensity.

Adenylic acids *a* and *b*, when separately heated under reflux in 80% acetic acid for 10 minutes, were both converted into a mixture of approximately equal parts of each. They were not interconverted by sodium hydroxide solution (0·25N.) at 28° or by aqueous ammonia (*d* 0·880) in a sealed tube at 100° during 24 hours. In the latter case some degradation to adenine and adenosine occurred.

Barium Salts of Adenosine Benzyl Hydrogen Phosphates a *and* b.—(i) 5′-Trityl adenosine dibenzyl phosphate (2·0 g.) was heated with acetic acid (8 c.c. of 80%) for 10 minutes and the product hydrogenated as before. In this case, hydrogenation was slow and incomplete. The

mixed barium salt of adenylic acids *a* and *b* was precipitated (0·363 g., 29%) by ethanol, and from the aqueous-ethanolic mother-liquors there was obtained, by concentration and further addition of ethanol, a crude barium salt. Further purification was effected by repeated precipitation from water with ethanol, but analytically pure material was not obtained. The product was a white hygroscopic powder (0·073 g.). Hydrolysis of this material with 0·1N-hydrochloric acid gave adenine, and micro-hydrogenation yielded a mixture of adenylic acids *a* and *b*, identified by paper chromatography; the material, on paper chromatograms, showed two components (R_P values, 0·56 and 0·67 in sodium phosphate and 0·44 and 0·50 in butanol–acetic acid: cf. Table).

(ii) To a suspension of adenylic acid *a* (0·016 g.) in pure, dry dimethylformamide (1·0 c.c.), phenyldiazomethane (0·23 c.c.) was added. The acid dissolved slowly with effervescence, and next morning the solution was examined on paper chromatograms. A component was present having similar R_P values in both sodium phosphate and butanol–acetic acid (0·67 and 0·44 respectively) to one of the components of the above benzyl esters. It is therefore designated adenosine benzyl hydrogen phosphate *a*. A similar reaction with adenylic acid *b* led to the identification of the other component (R_P 0·56 in sodium phosphate, 0·50 in butanol–acetic acid) as adenosine benzyl hydrogen phosphate *b*. Ether was added to the above dimethylformamide solutions, and the precipitated reaction products were washed with ether. In addition to the monobenzyl esters, small amounts of the dibenzyl esters also appeared to be present from a consideration of chromatograms. During the working up, some interconversion of the *a* and *b* monobenzyl esters also occurred.

The ether-washed product from a larger-scale preparation was shaken with chloroform and water, and the aqueous phase separated, washed with more chloroform, and then neutralised with barium hydroxide solution. After evaporation of the mixture to small bulk and filtration, ethanol precipitated the mixed barium salt of adenosine benzyl hydrogen phosphates *a* and *b* as a white hygroscopic powder (Found, in material dried at 113°/0·1 mm. over phosphoric oxide: C, 39·2; H, 4·1; N, 12·3. Calc. for $C_{17}H_{19}O_7N_5PBa_{\frac{1}{2}}$: C, 40·4; H, 3·8; N, 13·8. Calc. for $C_{17}H_{19}O_7N_5PBa_{\frac{1}{2}},H_2O$: C, 39·1; H, 4·0; N, 13·4%).

Hydrolysis of Adenosine Benzyl Hydrogen Phosphates a *and* b *with Acid and Alkali.*—Adenosine benzyl hydrogen phosphates *a* and *b* (2·7 mg.; from 5′-trityl adenosine dibenzyl phosphate) were dissolved in 80% acetic acid (0·2 c.c.), and the solution was heated at 100°. Paper chromatography using butanol–acetic acid showed that debenzylation to the adenylic acids was complete in 30 minutes. Some degradation to adenine also occurred. No debenzylation occurred when adenosine-5′ benzyl hydrogen phosphate was treated under the same conditions.

The mixed barium adenosine benzyl phosphates *a* and *b* (5·4 mg.; from 5′-trityl adenosine dibenzyl phosphate) was dissolved in sodium hydroxide solution (0·2 c.c. of 0·5N.) and set aside at 30° for 20 hours. Chromatographic analysis of this solution in both sodium phosphate and butanol–acetic acid solvents showed that complete conversion into a mixture of adenylic acids *a* and *b* had been effected.

In a similar reaction, the ether-precipitated and washed product (7·4 mg.) from the action of phenyldiazomethane on adenylic acid *b* was treated with sodium hydroxide solution (0·5 c.c. of 0·5N.; 50% aqueous ethanol). The product, characterised chromatographically in both solvents, was a mixture of adenylic acids *a* and *b*. An identical result was obtained on using the benzylated product from adenylic acid *a*.

Adenosine-5′ benzyl hydrogen phosphate (3 mg.) was treated as above with sodium hydroxide (0·3 c.c. of 0·5N.). Chromatography of the solution in butanol–acetic acid showed that the starting material was unaffected; no adenosine-5′ phosphate was produced.

One of us (D. M. B.) participated in this work during a period of leave of absence from the Chester Beatty Research Institute, Royal Cancer Hospital, London. Our thanks are offered to Roche Products Ltd. for grants and gifts of material.

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13. *Nucleotides. Part X.* Some Observations on the Structure and Chemical Behaviour of the Nucleic Acids.*

By D. M. Brown and A. R. Todd.

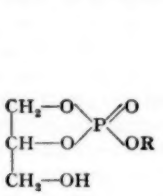
The chemistry of the isomeric mononucleotides obtained on hydrolysis of ribonucleic acids is reviewed and their behaviour is shown to parallel that of the glycerol monophosphates. This leads directly to an understanding of the hydrolytic behaviour of nucleic acids. The bearing of this and of other evidence on the problem of the general structure of the ribonucleic acids is discussed.

Since 1949 knowledge of the degradation products of the ribonucleic acids has undergone considerable change. Until that year it was believed that only four mononucleotides could be obtained by either chemical or enzymic hydrolysis of ribonucleic acids. These mononucleotides had been formulated as the 3′-phosphates of adenosine, guanosine, uridine, and cytidine in the light of degradative studies by Levene and Harris (*J. Biol. Chem.*, 1933, **101**, 419) on yeast adenylic and guanylic acids, the two pyrimidine nucleotides being tacitly assumed to have an analogous structure. The structures of the basic nucleosides had been completely established (see Lythgoe and Todd, *Nucleic Acid Symp. Soc. Exp. Biol.*, 1947, **1**, 15, Camb. Univ. Press, and numerous papers on nucleoside synthesis by Todd *et al.*, *J.*, 1943—1949). The fact that only four nucleotides could be isolated was a source of considerable difficulty when attempts were made to formulate the ribonucleic acids as polynucleotides in which individual nucleosides were joined together by phosphodiester linkages, and it led to various assumptions regarding the mode of linkage and reactivity towards hydrolytic agents which were difficult to accept or even to test experimentally (see Gulland, *J.*, 1938, 1722; 1944, 208; Tipson, *Adv. Carbohydrate Chem.*, 1945, **1**, 193; Schlenk, *Adv. Enzymology*, 1949, **9**, 455). Knowledge which has accrued since 1949 in other laboratories, taken in conjunction with our own investigations in the nucleotide field, has led to fresh considerations which appear to us to throw some light on the problems of the structure and chemical reactivity of the nucleic acids.

The separation of two isomeric adenylic acids from alkaline hydrolysates of yeast ribonucleic acid by Carter and Cohn (*Fed. Proc.*, 1949, **8**, 190, and subsequent papers) marked an important step forward in nucleic acid chemistry, and it was soon followed by the separation of the other three nucleotides into pairs of isomers (Cohn, *J. Amer. Chem. Soc.*, 1950, **72**, 1471, 2811; Loring, Luthy, Bortner, and Levy, *ibid.*, p. 2811). With respect to the isomeric adenylic acids, termed adenylic acids *a* and *b*, Carter (*ibid.*, p. 1466) suggested that these were adenosine-2′ and -3′ phosphate, the *b* isomer representing the long-known yeast adenylic acid, although he also considered α β-isomerism at the "*N*-glycosidic" linkage (position 1′) as a possible alternative explanation. That either of the isomers was adenosine-5′ phosphate was excluded on chemical and enzymatic grounds. Direct comparison of adenylic acid *a* with synthetic adenosine-2′ phosphate was impossible because synthetic compounds hitherto regarded as nucleoside-2′ phosphates were shown not to have this structure, the benzylidene nucleosides used in their preparation being 2′ : 3′-benzylidene derivatives and not 3′ : 5′- as had been earlier believed (Brown, Haynes, and Todd, *J.*, 1950, 2299). Moreover, since benzylidene adenosine had been used as an intermediate in the synthesis of yeast adenylic acid (Michelson and Todd, *J.*, 1949, 2476) the identification of adenylic acid *b* with this material did not prove that the phosphate residue was attached to $C_{(3')}$; the synthetic method was ambiguous in the sense that the phosphate group in the final product could be either at $C_{(2')}$ or $C_{(3')}$. We have already (Brown and Todd, preceding paper) presented our evidence for the view that adenylic acids *a* and *b* are the 2′- and the 3′-phosphates of adenosine, although not necessarily respectively, and that they are identical with the two isomeric nucleotides obtained by phosphorylating 5′-trityl adenosine.

* Part IX, preceding paper.

The adenylic acids *a* and *b* are readily converted into a mixture of each under acid conditions (Brown, Haynes, and Todd, *loc. cit.*), and the same observation has been made on the isomeric pyrimidine nucleotides (Cohn, *J. Amer. Chem. Soc.*, 1950, **72**, 2811). On the other hand, the isomers are stable, without interconversion, under alkaline conditions (Brown and Todd, *loc. cit.*). These experiences find their counterpart in the chemistry of the glycerol phosphates. Thus, glycerol α-phosphate, which is unaffected by alkali, is rearranged in warm dilute acid solution to an equilibrium mixture of glycerol α- and β-phosphate (Bailly, *Compt. rend.*, 1938, **206**, 1902; 1939, **208**, 443, 1820; Verkade, Stoppelenburg, and Cohen, *Rec. Trav. chim.*, 1940, **59**, 886). This rearrangement has been shown to be intramolecular (Chargaff, *J. Biol. Chem.*, 1942, **145**, 455) and it has also been observed with the phosphatidic acids (Baer and Kates, *J. Biol. Chem.*, 1950, **185**, 615). Thus far the isomeric nucleotides and the glycerol phosphates appear to be analogous. In the latter series, it was postulated (Verkade *et al.*, *loc. cit.*; Chargaff, *loc. cit.*) that the phosphoryl migration occurred by way of an intermediate cyclic phosphate ester (I; R = H), although the substance itself was not isolated. We assumed that the isomerisation of the adenylic acids involved the cyclic intermediate (II). Work in progress in these laboratories (with Mr. D. Magrath) has shown that this and other cyclic nucleoside-2′ : 3′ phosphates are accessible substances and that they break down readily on hydrolysis to give a mixture of the 2′- and the 3′-phosphates.



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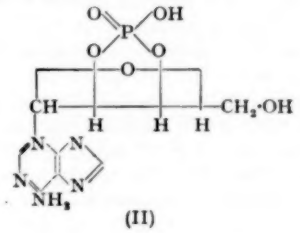

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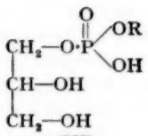

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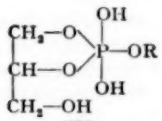
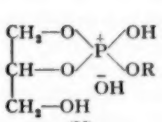


(IV)



(V)

Some further points in the chemistry of the glycerol phosphates deserve mention. Bailly and Gaumé (*Bull. Soc. chim.*, 1935, **2**, 354) showed that whereas glycerol α-phosphate is stable to alkali, glycerol α-(methyl hydrogen phosphate) (III; R = Me) is readily hydrolysed under alkaline conditions to methanol and a mixture of glycerol α- and β-phosphate; no methyl phosphate is produced. Baer and Kates have described a similar hydrolysis of the choline phosphate (III; R = choline residue); choline was liberated and a mixture of glycerol α- and β-phosphate resulted (*J. Biol. Chem.*, 1948, **175**, 79). Alkaline degradation of the lecithins results in phosphoryl migration (Baer and Kates, *ibid.*, 1950, **185**, 615). Presumably the same factor operates in the case of 2-hydroxyethyl dimethyl phosphate which, under the action of dilute alkali, forms 2-hydroxyethyl phosphate with loss of methanol (Bailly and Gaumé, *Bull. Soc. chim.*, 1936, **3**, 1396). It is noteworthy that 2-methoxyethyl dimethyl phosphate is hydrolysed by dilute alkali to 2-methoxyethyl methyl hydrogen phosphate which is stable towards alkali, a result which is in accord with the generally recognised stability of dialkyl esters of phosphoric acid toward alkaline reagents (cf. Kosolapoff, "Organo-Phosphorus Compounds," Wiley, New York, 1950, p. 232).

It thus appears that the prerequisite for alkali lability in dialkyl phosphates is the presence of a hydroxyl function in proximity to the phosphoryl group. That interaction between neighbouring hydroxyl and phosphoryl groups takes place has been stressed by Kumler and Eiler (*J. Amer. Chem. Soc.*, 1943, **65**, 2355) who have shown that the polyol

and sugar phosphates are abnormally strong acids in comparison with the monoalkyl phosphates.

It is well known that the triesters of phosphoric acid are sensitive both to alkali and to acid (Kosolapoff, *op. cit.*). We envisage a mechanism for the hydrolysis of the hydroxylated dialkyl phosphates in which a neutral cyclic phospho-triester is involved. Thus, in the alkaline hydrolysis of glycerol α-(methyl hydrogen phosphate), (I; R = Me) is produced and immediately hydrolysed to methanol and the cyclic phosphate (I; R = H) which then gives rise to glycerol α- and β-phosphate. No other major products are to be expected, since it is evident that, although the three ester linkages in the cyclic triester must be of comparable reactivity, yet only by fission of the bond retaining the singly-linked substituent can degradation of the molecule occur. Baer and Kates (*J. Biol. Chem.*, 1948, **175**, 79) had earlier proposed a cyclic orthophosphate intermediate (IV; R = choline residue) in the alkaline fission of the choline glycerol α-phosphate. The phosphonium structure (V) may also be considered.

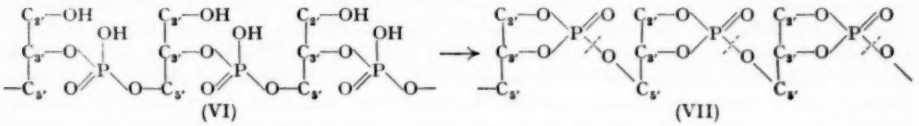
Fonó (*Arkiv Kemi, Min., Geol.*, 1947, **24**, *A*, No. 33, pp. 14, 15) has already pointed out the importance of the neighbouring hydroxyl groups in causing lability of diesters of phosphoric acid containing a glycerol or ethylene glycol residue and has also postulated the existence of a cyclic triester intermediate in their hydrolysis. In this important though little quoted paper he also advanced the view that the alkali-lability of ribonucleic acid, in contrast to the alkali-stability of deoxyribonucleic acid, may be attributed to the extra hydroxy-group present at $C_{(2)}$ in the sugar residues of the former, *i.e.*, that ribonucleic acids are analogous to glycerol alkyl phosphates in their hydrolytic behaviour. On the evidence available to him at the time of his paper, however, he was unable to develop the theme further, nor did he appear to appreciate fully its implications.

During our synthetic experiments we have had occasion to hydrolyse 5′-trityl adenosine-2′ (and -3′) dibenzyl phosphate with 80% acetic acid (Bredereck, Berger, and Ehrenberg, *Ber.*, 1940, **73**, 269) to effect the removal of the trityl and one benzyl group. It is known that monodebenzylation takes place smoothly under comparable conditions in derivatives of adenosine-5′ dibenzyl phosphate (Baddiley and Todd, *J.*, 1947, 648; Brown, Haynes, and Todd, *loc. cit.*). We found that a considerable proportion of nucleotide (adenylic acids *a* and *b*) was produced, involving the loss of both benzyl groups. Furthermore, when a mixture of adenosine-2′ and -3′ benzyl hydrogen phosphate (Brown and Todd, *loc. cit.*) was set aside with 0·5N-sodium hydroxide at 30° overnight, complete debenzylation to a mixture of adenylic acids *a* and *b* occurred. Under the same conditions adenosine-5′ benzyl hydrogen phosphate was quite unaffected. The reaction between phenyldiazomethane and adenylic acids *a* and *b* affords a mixture of the dibenzyl and monobenzyl esters of the respective acids; these esters are each degraded, with rearrangement, to a mixture of adenylic acids *a* and *b* by dilute sodium hydroxide. These experiments show that complete accord exists between the reactions of the adenylic acid derivatives and those of the glycerol phosphates.

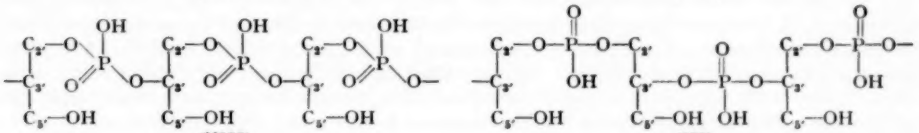
The above considerations can be applied directly to the polynucleotides. It has long been known that ribonucleic acids suffer rapid degradation to their component mononucleotides under a variety of mildly alkaline conditions (Steudel and Peiser, *Z. physiol. Chem.*, 1922, **120**, 292; Levene, *J. Biol. Chem.*, 1919, **40**, 415; 1923, **55**, 9; Jones and Perkins, *ibid.*, 1925, **62**, 557; Calvery, *ibid.*, 1927, **72**, 27; Loring, Roll, and Pierce, *ibid.*, 1948, **174**, 729). In none of these cases has any fission product larger than a mononucleotide been isolated; claims to the isolation of larger fragments (Jones *et al.*, *ibid.*, 1916, **25**, 93; 1917, **29**, 111, 123; **31**, 39; Thannhauser and Dorfmüller, *Z. physiol. Chem.*, 1915, **95**, 259) have generally been relinquished or refuted by other workers on the general grounds that the products described were separable mixtures of mononucleotides (Levene, *J. Biol. Chem.*, 1919, **33**, 229). In the same way, mild acid hydrolysis of ribonucleic acid yields mononucleotides, although further degradation, especially of the purine nucleotides, occurs. The early work in this field was hindered by difficulties in separation and identification of the products, but latterly application of chromatographic analysis, together with physical methods of identification, have amply confirmed the earlier observations.

Deoxyribonucleic acids are not degraded to small molecules by mild treatment with alkali (Steudel and Peiser, *loc. cit.*); this observation has provided a method for their analytical separation from ribonucleic acids (Schmidt and Thannhauser, *J. Biol. Chem.*, **1945**, **161**, 83).

If the simple straight-chain polynucleotide sequence (VI) in which the individual nucleoside residues are represented briefly as $C_{2'}$–$C_{3'}$–$C_{5'}$ be considered, it can be seen that alkaline degradation will proceed through an intermediate (VII), followed by ready fission of the triester groups *exclusively* at the P–O–$C_{5'}$ linkage. The liberated cyclic nucleoside-2′ : 3′ phosphates will then give rise to a mixture of nucleoside-2′ and -3′ phosphates. No



(VI) (VII)



(VIII) (IX)

such mechanism can apply in the case of the deoxyribonucleic acids where the absence of a hydroxyl at $C_{2'}$ precludes the essential formation of a cyclic structure, and this accounts for their more normal stability towards alkali and acid. The isolation of thymidine diphosphate and cytosine deoxyriboside diphosphate after relatively vigorous acid hydrolysis of deoxyribonucleic acid (Levene and Jacobs, *J. Biol. Chem.*, 1912, **12**, 411; 1938, **126**, 63; Thannhauser and Ottenstein, *Z. physiol. Chem.*, 1921, **114**, 39; Dekker and Todd, unpublished observations) may be regarded as confirmation of our view that no special mechanism operates in this case and that the fission of the polynucleotide proceeds in accordance with the known behaviour of phosphodiesters towards acids (Kosolapoff, *op. cit.*); analogous ribonucleoside diphosphates have never been obtained from ribonucleic acids by chemical hydrolysis, nor are they, in our view, to be expected.

An understanding of the mechanism of alkaline hydrolysis of ribonucleic acid does not of itself define the position of the internucleotide linkages, although it excludes several possible combinations. In addition to structure (VI) in which the polymeric linkage is shown joining the 3′- and 5′-positions of the nucleoside residues, other structures containing a $C_{(2')}$–$C_{(5')}$ or $C_{(2')}$–$C_{(3')}$ (VIII) repeating unit or mixed $C_{(2')}$–$C_{(2')}$ and $C_{(3')}$–$C_{(3')}$ units in sequence (IX) would all show lability to alkali on the basis of the mechanism under discussion. A $C_{(5')}$–$C_{(5')}$ linkage, however, cannot occur anywhere in the molecule as this would be stable to alkali and would lead to the appearance of dinucleotides in ribonucleic acid hydrolysates. Gulland and Smith (*J.*, 1948, 1532) prepared a compound which they considered to be diuridine-2′ : 2′ phosphate and which was stable to alkali and acid under conditions which bring about hydrolysis of yeast ribonucleic acid. The synthetic route used in preparing this substance has since been shown (Brown, Haynes, and Todd, *loc. cit.*) to yield 5′-substituted nucleosides so that the compound of Gulland and Smith was in reality diuridine-5′ : 5′ phosphate. Its stability is therefore in accord with our expectation that the cyclisation necessary for rearrangement and easy fission cannot occur between the 5′- and either the 2′- or the 3′-position in the same ribose residue. Consideration of the stereochemistry of the ribofuranoside ring structure suggests that such a cyclisation would not be likely to occur, although Gulland (*J.*, 1938, 1722) at one time considered the possibility of a $C_{(5')} \longrightarrow C_{(3')}$ migration in seeking to explain the non-occurrence of 5′-nucleotides in chemical hydrolysates of ribonucleic acids in face of evidence for 5′-phosphoester linkages from enzyme experiments. One difference between the poly-3′ : 5′-diester structure (VI) and structures (VIII) and (IX) is that in (VI) rupture

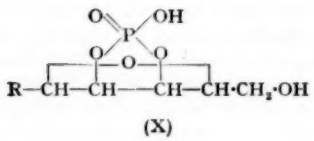
of the chain to yield mononucleotides could, in theory, occur simultaneously at many points, whereas in (VIII) and (IX) it must occur stepwise starting from the end of the chain bearing a free hydroxyl at $C_{(2')}$ or $C_{(3')}$. Such considerations will, of course, only apply to hypothetical unbranched polynucleotide chains and would have to be modified in, for example, branched-chain structures based on (VI).

Mainly as a result of experiments using enzymes, evidence for the occurrence of 5′-phosphoester linkages in nucleic acids has gradually accumulated, although general acceptance of their presence was prevented in the past by the complete failure, until recently, of attempts to isolate nucleoside-5′ phosphates from ribonucleic acid hydrolysates. The evidence rested upon the action of phosphatases, in particular the specific 5′-nucleotidase (Takahashi, *J. Biochem. Japan*, 1932, **16**, 463; Klein and Rossi, *Z. physiol. Chem.*, 1935, **236**, 201; Gulland and Jackson, *J.*, 1938, 1492). The enzyme preparations used have in general contained more than one component. The Russell's viper venom used by Gulland and Jackson had in addition to 5′-nucleotidase and phosphodiesterase activity also a weak non-specific phosphomonoesterase activity; however, they showed that acting on yeast ribonucleic acid the venom alone liberated 25% of the bound phosphorus as inorganic phosphate and this was increased to 75% by the addition of bone phosphomonoesterase. These earlier indications of 5′-phosphoester linkages have now been confirmed by Cohn and Volkin (*Nature*, 1951, **167**, 483) who treated ribonucleic acid successively with ribonuclease and intestinal phosphatase in presence of arsenate to inhibit the phosphomonoesterase. The enzymic hydrolysate was analysed chromatographically on an ion-exchange resin and shown to contain large amounts of the 5′-phosphates of adenosine, guanosine, uridine, and cytidine, identified by direct comparison with synthetic specimens (Michelson and Todd, *loc. cit.*). Using the same phosphatase system (Klein, *Z. physiol. Chem.*, 1933, **218**, 164) Klein and Thannhauser (*ibid.*, p. 173; 1934, **224**, 252; 1935, **231**, 96) and Volkin, Khym, and Cohn (*J. Amer. Chem. Soc.*, 1951, **73**, 1533) have isolated four deoxyribonucleotides from deoxyribonucleic acid. Experiments with purified 5′-nucleotidase (Heppel and Hilmoe, *J. Biol. Chem.*, 1951, **188**, 665) show that these are almost certainly 5′-deoxyribonucleotides (Carter, *J. Amer. Chem. Soc.*, 1951, **73**, 1573). Further evidence for the existence of 5′-phosphoester groupings in ribonucleic acids comes from the observations of Allen (*Fed. Proc.*, 1951, **10**, 155) and of Cavalieri, Kerr, and Angelos (*J. Amer. Chem. Soc.*, 1951, **73**, 2567) on the periodate titration of products obtained by treatment of ribonucleic acid with ribonuclease.

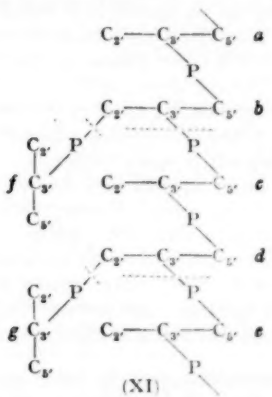
On the available evidence, then, there can be no doubt that $C_{(5')}$ is involved in the main internucleotide linkage both of ribonucleic and of deoxyribonucleic acids. In the case of the latter, the main linkage must be the $C_{(3')}$–$C_{(5')}$, but a decision between $C_{(2')}$ and $C_{(3')}$ as the second point of attachment in ribonucleic acids cannot yet be made with certainty. It is hoped that further evidence on this point will accrue from a study of dinucleoside phosphates and dinucleotides at present being prepared in this laboratory. The production of both nucleoside-2′ and -3′ phosphates on alkaline hydrolysis and the interconversion possibility precludes this method, at least in its present form, from yielding information as to whether $C_{(2')}$ or $C_{(3')}$ or both positions are involved along with $C_{(5')}$. It should, moreover, be remembered that the designations *a* and *b* for the isomeric adenine mononucleotides were arbitrarily chosen and we do not know with certainty which is a 2′- and which a 3′-phosphate. At the moment, however, it seems advantageous to identify the second point of attachment of the main internucleotide linkages in ribonucleic acids as $C_{(3')}$, *i.e.*, to regard the "backbone" of the molecule as a sequence of nucleosides linked together by phosphoric acid groups between $C_{(3')}$ and $C_{(5')}$ as in (VI) by analogy with deoxyribonucleic acids where a $C_{(2')}$ link cannot occur and by consideration of the physical evidence, admittedly small (cf. Astbury, *Nucleic Acid Symp. Soc. Exp. Biol.*, 1947, **1**, 66). Such a structure would, on the arguments developed in this memoir, be hydrolysed readily in alkali to a mixture of isomeric 2′- and 3′-nucleotides, and with enzymes preferential rupture of the secondary alkyl linkages, *i.e.*, at P–O–$C_{(3')}$ rather than P–O–$C_{(5')}$, would yield 5′-nucleotides as it does with the deoxyribonucleic acids. It is, of course, clear that in the discussion which follows the same would hold good for precisely similar structures in which $C_{(2')}$ and $C_{(3')}$ were interchanged.

Although deoxyribonucleic acids appear to be largely straight-chain polynucleotides, many lines of evidence indicate that ribonucleic acids have a branched-chain structure. Electrometric titration data have been interpreted by invoking branched structures involving in particular phosphotriester groups to account for the presence of secondary phosphoryl dissociations (Fletcher, Gulland, and Jordan, *J.*, 1944, **33**; Gulland and Jordan, *Nature*, 1948, **161**, 561; Cavalieri, Kerr, and Angelos, *loc. cit.*). There is nevertheless no general agreement as to the proportion of monoesterified phosphate in the nucleic acids studied (Zittle, *J. Biol. Chem.*, 1946, **166**, 491; Allen and Eiler, *ibid.*, 1941, **137**, 757; Wiener, Duggan, and Allen, *ibid.*, 1950, **185**, 163; Vandendriessche, *Compt. rend. Trav. Lab. Carlsberg*, 1951, **27**, 341). The action of ribonuclease on ribonucleic acids gives rise to fragments of widely varying molecular weight (and of which up to 85% may be dialysable) without liberation of inorganic phosphate. Schmidt, Cubiles, Swartz, and Thannhauser (*J. Biol. Chem.* 1947, **170**, 759) found that in addition to larger fragments only pyrimidine nucleotides are liberated by ribonuclease, an observation which has been confirmed by Carter and Cohn (*J. Amer. Chem. Soc.*, 1950, **72**, 2604). Loring and Carpenter (*J. Biol. Chem.*, 1943, **150**, 381) obtained both purine and pyrimidine nucleotides from a nucleic acid in this way. Markham and Smith (*Research*, 1951, **4**, 344) report that a large proportion of the pyrimidine nucleotide present in turnip yellow mosaic virus ribonucleic acid and other ribonucleic acids is liberated in two fractions by ribonuclease in about 10 minutes and that these fractions, by chemical hydrolysis or by the further action of ribonuclease, yield uridylic and cytidylic acids respectively. These two fractions have been provisionally identified by paper chromatography with the cyclic 2′ : 3′-phosphates of uridine (X; R = uracil) and cytidine (X; R = cytosine) prepared by Mr. D. Magrath in this laboratory; further experiments are in progress in collaboration with Drs. Markham and Smith to confirm the identity.

This production of large amounts of pyrimidine nucleotides following ribonuclease treatment of ribonucleic acids suggests that these nucleotides are derived from side chains in the molecule and that these chains or branches must occur at frequent intervals. It suggests further that the side-chains are short—probably for the most part containing only one nucleotide residue. If we consider a backbone structure (VI) for the



(X)



(XI)

nucleic acid, then, omitting for the moment branching by inclusion of triester linkages, it is clear that the attachment of the branches to the main chain must occur at $C_{(2')}$ and that this must be linked through the phosphate group to $C_{(3')}$ (or $C_{(2')}$) in the first nucleoside residue in any branch. Only by such means could the alkali-lability of the nucleic acid be maintained; linkage of $C_{(2')}$ in the main chain to $C_{(5')}$ in the first residue of the side-chain would give an alkali-stable structure (since cyclisation would be impossible in such a structure, *both* $C_{(2')}$ and $C_{(3')}$ in the nucleoside residue in the main chain at the branching point being occupied). The general structure which we envisage for ribonucleic acids is that shown diagrammatically in (XI).

In this structure, the branches are shown as containing one nucleotide only; longer chains, if they occur, would be accommodated by extending them by the normal $C_{(5')}$–$C_{(3')}$ sequence as in the main chain. The attachment of a certain number of branches on phosphorus, *i.e.*, the incorporation of phosphotriester linkages, would present no problem as the grouping involved would be alkali-labile, provided, again, that the $C_{(5')}$–$C_{(3')}$ sequence were followed in the branching chain. Dilute alkali would convert a molecule of type (XI) into a mixture of the mononucleotides in accordance with the observed facts. If we assume that *f* and *g* are pyrimidine nucleoside residues, then their removal as a first step in ribonuclease action by fission at the P–O–$C_{(2')}$ linkage (probably initially as cyclic phosphates) is in accordance with our prediction. The increased periodate uptake shown by ribonuclease-treated ribonucleic acid already mentioned would be explained by simultaneous or subsequent fission of some of the $C_{(3')}$–O–P linkages in the main chain, as indicated by the broken line in (XI). It will be observed (*a*) that fragments would be produced bearing at one end a nucleoside residue attached by phosphate through the 5′-position only, *i.e.*, oxidisable by periodate, (*b*) that subsequent treatment of the products with alkali should yield in addition to mononucleotides a quantity of free nucleosides, and (*c*) that, although they have not as yet been isolated, some nucleoside diphosphates should occur in *alkaline* hydrolysates of ribonuclease-treated ribonucleic acids. It will further be noted that enzymic hydrolysis of such a structure as (XI) with ribonuclease followed by intestinal phosphatase should yield the four 5′-nucleotides from the main chain, and in addition the side chain groups *f* and *g* should yield 2′- or 3′-nucleotides. It is of considerable interest that in addition to 5′-nucleotides and some unidentified materials Cohn and Volkin (*Nature*, *loc. cit.*; *Fed. Proc.*, 1951, **10**, 173) have shown that such treatment does give also the *b* isomers of uridylic and cytidylic acids in considerable amount. Present knowledge of ribonuclease specificity does not, of course, permit the conclusion that in the main or "backbone" chain of (XI) only $C_{(3')}$–O–P linkages would undergo fission by its action; $C_{(5')}$–O–P linkages may also be split without affecting our general thesis. The $C_{(3')}$–O–P fission has been singled out for special comment above because, only if it does occur, can immediate increase in periodate uptake following ribonuclease action be explained.

There can be no question of finality about any nucleic acid structure at the present time, since it is clear that there is no available method for determining the nucleotide sequence. If the view of Schmidt and Thannhauser (Ann. Report, Nat. Res. Council Comm. on Growth, U.S.A., 1950, 194) be accepted that ribonuclease attacks pyrimidine nucleotide linkages preferentially, then it is possible to speculate as to the location of pyrimidine nucleotides in the main polynucleotide chain [*e.g.*, *b* and *d* in (XI)] but such speculations go rather beyond present evidence. There are, too, some possible variants of structure (XI) which could equally be considered since the choice between $C_{(2')}$ and $C_{(3')}$ as a linking point in any given residue is arbitrary. Nevertheless, we feel it desirable to indicate that structures of type (XI) are capable of explaining a surprising number of the known facts about the hydrolytic behaviour of ribonucleic acids, without invoking any linkages between nucleoside units other than simple phosphate groups between hydroxyl groups in the ribofuranose residues. At present they represent a convenient working hypothesis which we hope to test in further synthetic studies now in progress.

We acknowledge gratefully many helpful discussions with our colleagues in this laboratory, in particular Dr. C. A. Dekker.

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14. *The Oxidation of Monoethenoid Fatty Acids and Esters. Union of Gaseous Oxygen with Ethyl, n-Propyl, and n-Butyl Oleate.*

By A. J. Feuell and J. H. Skellon.

The catalytic oxidation at 120°, 85°, and 55° of ethyl, *n*-propyl, and *n*-butyl oleate has been studied. The results show that peroxide formation and subsequent decomposition are a characteristic feature of autoxidations at the higher temperatures. Changes in iodine and saponification values are also characteristic for each temperature. Prolonged oxidation at 120° produces some dimerisation together with formation of reducing substances. The nature of the terminal alkyl group influences the course of the reaction, maximum peroxide formation decreasing progressively from ethyl to *n*-butyl. Comparison with previous work indicates that the configuration of the monoethenoid acid molecule is an important factor, the (*cis*-)oleic esters reacting more rapidly than the corresponding (*trans*-)elaidic esters.

Considerable attention has been given in recent years to the oxidation of oleic acid and its methyl ester (see, *e.g.*, Skellon and Gordon, *Chem. and Ind.*, 1951, 629, for a review), but the autoxidation of the higher esters has been little studied. The oxidation of ethyl oleate was examined chemically by Armstrong and Hilditch (*J. Soc. Chem. Ind.*, 1925, **44**, 43T) and spectrochemically by Lundberg, Holman, and Burr (*Oil and Soap*, 1946, **23**, 10), and the oxidation of butyl oleate by Deatherage and Matill (*Ind. Eng. Chem.*, 1939, **31**, 1425).

The object of the present work was to compare the oxidation of the higher esters of oleic acid in the presence of a metallic catalyst with similar catalytic autoxidations of the methyl ester (see, *e.g.*, Skellon, *J.*, 1948, 343), especially in regard to the influence of the terminal alkyl group on the ease of entry of oxygen into the monoethenoid acid molecule.

Pure oleic acid was prepared by a combination of lead and lithium salting as previously described (Skellon, *J. Soc. Chem. Ind.*, 1931, **50**, 131T) and converted into its esters by a method based on that of Armstrong and Hilditch (*loc. cit.*). Uranium oleate (Skellon and Spence, *ibid.*, 1948, **67**, 365) was employed as the catalyst, it having been shown (Skellon, *ibid.*, 1950, **69**, 116) that uranium soaps are among the most effective autoxidation catalysts. The conditions of oxidation were such as to give high interim peroxide formation with minimum of fission or polymerisation.

For all esters, the highest peroxide values were obtained at 85° after 20—26 hours (Fig. 1), *viz.*, ethyl ester 1·50, *n*-propyl 1·23, *n*-butyl 1·16% (peroxide values expressed as % of active oxygen). In comparison, Gunstone and Hilditch (*J.*, 1945, 836) obtained for methyl oleate at 80° a maximum peroxide content of 1·95% after 68 hours. In all cases, the observed peroxidic oxygen content was below the theoretical maximum (calc. for esters : ethyl 4·67, *n*-propyl 4·49, *n*-butyl 4·32%) ; and it is evident from the curves that the peroxides are unstable, decomposing more or less rapidly to give secondary products.

At 120°, the rapid increase of peroxidation followed by a somewhat comparable rate of decrease was similar to that observed for methyl oleate by Gunstone and Hilditch (*loc. cit.*) and by Skellon (*J.*, 1948, 343), and for methyl and *n*-propyl elaidate by Skellon and Thruston (*J.*, 1949, 1626). The peroxide maxima were lower at this temperature, *viz.*, ethyl ester, 0·94, *n*-propyl 0·87, *n*-butyl 0·38%, but were attained much more rapidly (3—4 hours). Comparable data for methyl oleate are given by Gunstone and Hilditch (*loc. cit.*) who reported a peroxide maximum of 1·17% after 4 hours, and by Skellon (*loc. cit.*) who reported a maximum of 0·76% after 6 hours using a cobalt oleate catalyst.

The decreases in iodine value (Fig. 2) and increases in saponification value (Fig. 3) are characteristic for each of the three temperatures, and obviously suggest differences in the mode of catalytic oxidation under varying thermal conditions, in agreement with observations of the various investigators cited above.

The final products resulting from the 120° oxidations all gave positive and rapid reactions in qualitative tests for reducing agents, suggesting the presence of carbonyl compounds, whilst the increased molecular weights indicated the onset of some form of

FIG. 1.

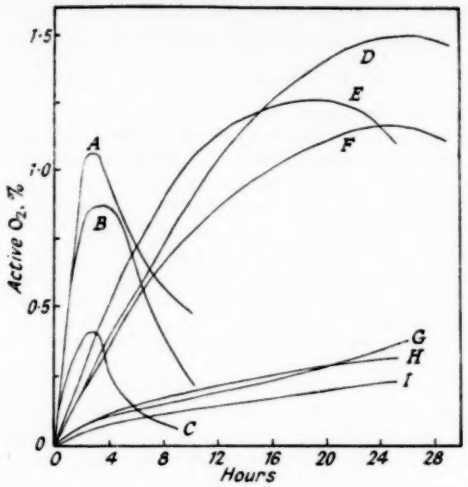


FIG. 2.

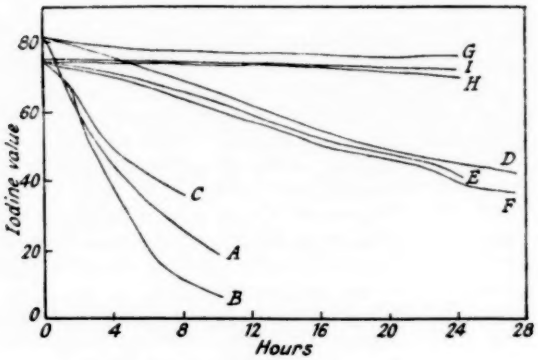
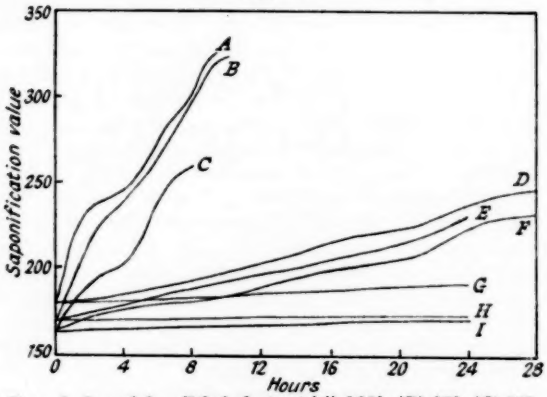


FIG. 3.



FIGS. 1, 2, and 3. *Ethyl oleate at* (*A*) 120°, (*D*) 85°, (*G*) 55°.
n-*Propyl oleate at* (*B*) 120°, (*E*) 85°, (*H*) 55°.
n-*Butyl oleate at* (*C*) 120°, (*F*) 85°, (*I*) 55°.

polymerisation. At 85°, the final products gave only slight reactions for reducing substances; but the decreased molecular weights pointed to some degree of fission. At 55°, little change was observed even after 24 hours' oxidation.

Ease of entry of oxygen into the monoethenoid acid molecule is clearly influenced by the size of the terminal alkyl group, a progressive decline in reactivity being apparent as the homologous series is ascended from ethyl to *n*-butyl. A similar effect was noted by Skellon and Thruston (*loc. cit.*) for methyl and *n*-propyl elaidate.

Finally, an interesting series of comparisons may be made with the results obtained, under very similar conditions of catalytic oxidation, for the methyl and *n*-propyl esters of the corresponding *trans*-isomer, elaidic acid (Skellon and Thruston, *loc. cit.*). The most notable feature is that although the *trans*-esters attained similar peroxide maxima, these were reached only after a considerably longer period: *e.g.*, at 85°, *n*-propyl elaidate required 42—48 hours compared with 20—22 hours for *n*-propyl oleate; at 120°, the two *trans*-esters required 6—10 hours compared with 3—4 hours for the three *cis*-esters. The configuration of the molecule thus appears to affect the rate of formation and decomposition of peroxides in thermal oxidations.

The results of detailed examination of the oxidation products of the esters at various stages will form the subject of further communications.

Experimental

Oleic Acid.—Pure oleic acid was prepared from high-grade olive oil (1·5 kg.), by a lead and lithium salting method (*J. Soc. Chem. Ind.*, 1931, **50**, 131T), in a yield of 300 g., as an almost colourless oil, n_D^{20} 1·4603 (Found: Sap. val., 197·2; I val., 90·0. Calc. for $C_{18}H_{34}O_2$: Sap. val., 198·6; I val., 89·9).

Esters.—Oleic acid (80 g.), the appropriate alcohol (160 g.), and concentrated sulphuric acid (4 ml.) were refluxed for 2 hours. Excess of alcohol was distilled off and the residue poured into water (400 ml.) and extracted with ether. The ethereal extracts were washed with dilute sodium carbonate solution and dried (Na_2SO_4). After filtration, the ether was removed under reduced pressure, giving 80—85 g. of almost colourless liquids with the following characteristics: Ethyl oleate, n_D^{20} 1·4502 (Found: Sap. val., 181·2; I val., 80·3. Calc. for $C_{20}H_{38}O_2$: Sap. val., 180·8; I val., 81·7); *n*-propyl oleate, n_D^{20} 1·4500 (Found: Sap. val., 170·2; I val., 74·3. Calc. for $C_{21}H_{40}O_2$: Sap. val., 172·9; I val., 78·2); *n*-butyl oleate, n_D^{20} 1·4550 (Found: Sap. val., 165·5; I val., 75·8. Calc. for $C_{22}H_{42}O_2$: Sap. val., 165·7; I val., 75·0).

Catalytic Oxidations.—The ester (25 g.) was mixed with uranium oleate (0·05 g.) previously dissolved in a few ml. of the ester by warming. The uranium oleate was prepared by the

Catalytic oxidation of ethyl oleate.

At 120°.

Time (hours)	2	4	6	8	10		
Peroxide value	1·044	0·939	0·674	0·571	0·488		
I value	57·9	46·7	34·4	29·5	19·5		
Sap. value	235	244	276	294	323		
M (Rast) *	—	—	—	—	328		
n_D^{20}	1·4541	1·4554	1·4561	1·4573	1·4588		
Reduction of Fehling's soln. and ammoniacal $AgNO_3$							Rapid

At 85°.

Time (hours)	4	8	12	16	20	24	28
Peroxide value	0·329	0·751	1·034	1·246	1·426	1·480	1·476
I value	76·4	68·7	62·1	54·1	50·1	46·3	40·7
Sap. value	186	193	202	217	221	238	244
M (Rast) *	—	—	—	—	—	—	257
n_D^{20}	1·4520	1·4530	1·4539	1·4547	1·4552	1·4560	1·4562
Reduction of Fehling's soln. and ammoniacal $AgNO_3$							Slight

At 55°.

Time (hours)	4	8	12	16	20	24	
Peroxide value	0·102	0·124	0·187	0·239	0·277	0·341	
I value	78·5	77·5	76·5	76·0	75·8	76·0	
Sap. value	183	184	185	186	189	191	
M (Rast) *	—	—	—	—	—	343	
n_D^{20}	1·4515	1·4519	1·4521	1·4522	1·4523	1·4524	
Reduction of Fehling's soln. and ammoniacal $AgNO_3$							None

* Calc. for $C_{20}H_{38}O_2$: *M*, 310·5.

method of Skellon and Spence (*J. Soc. Chem. Ind.*, 1948, **67**, 365) (Found: U, 27·6. Calc.: U, 28·6%). The oxidation was carried out in an apparatus essentially similar to that used by Skellon and Thruston (*J.*, 1949, 1626), except that temperature control (±1°) was by means of a thermostatic air-oven. As the oxidations at 120° and 85° were exothermic in the initial stages it was necessary to keep the ambient temperature 5—10° below the required reaction temperature. The oxygen rate was 50 ml. per minute (approx.), and the gas emerged as a fine stream of bubbles from the delivery tube. The stirring rate was 300 r.p.m. (approx.). Samples were withdrawn by a fine pipette at suitable intervals.

The oxidations were conducted at 55°, 85°, and 120°. Changes studied are recorded in the Tables, peroxide values being expressed as % of active oxygen (method of Skellon and Wills, *Analyst*, 1948, **73**, 78). The course of the various oxidations is shown by the curves of Figs. 1, 2, and 3.

Catalytic oxidation of n-*propyl oleate.*

At 120°.

Time (hours)	2	4	6	8	10	
Peroxide value	0·821	0·864	0·671	0·387	0·231	
I value	56·3	36·9	17·8	11·9	7·5	
Sap. value	217	236	264	303	325	
M (Rast) *	—	—	—	—	365	
n_D^{20}	1·4543	1·4551	1·4560	1·4564	1·4577	
Reduction of Fehling's soln. and ammoniacal $AgNO_3$						Rapid

At 85°.

Time (hours)	4	8	12	16	20	24	
Peroxide value	0·524	0·904	1·150	1·230	1·232	1·104	
I value	71·8	66·2	58·3	50·7	47·6	39·2	
Sap. value	178	190	195	205	215	232	
M (Rast) *	—	—	—	—	—	263	
n_D^{20}	1·4521	1·4535	1·4541	1·4547	1·4550	1·4553	
Reduction of Fehling's soln. and ammoniacal $AgNO_3$							Slight

At 55°.

Time (hours)	4	8	12	16	20	24	
Peroxide value	0·106	0·147	0·201	0·253	0·288	0·312	
I value	74·5	74·1	72·0	71·3	69·8	69·5	
Sap. value	171	172	172	173	174	173	
M (Rast) *	—	—	—	—	—	327	
n_D^{20}	1·4508	1·4518	1·4522	1·4523	1·4527	1·4530	
Reduction of Fehling's soln. and ammoniacal $AgNO_3$							None

* Calc. for $C_{21}H_{40}O_2$: *M*, 324·5.

Catalytic oxidation of n-*butyl oleate.*

At 120°.

Time (hours)	2	4	6	8	
Peroxide value	0·331	0·219	0·120	0·085	
I value	62·1	49·1	43·4	36·1	
Sap. value	192	201	243	257	
M (Rast) *	—	—	—	414	
n_D^{20}	1·4568	1·4583	1·4603	1·4620	
Reduction of Fehling's soln. and ammoniacal $AgNO_3$					Rapid

At 85°.

Time (hours)	4	8	12	16	20	24	28
Peroxide value	0·375	0·633	0·851	0·967	1·059	1·160	1·103
I value	72·5	63·7	58·6	51·6	46·7	39·9	38·0
Sap. value	177	180	190	197	205	226	231
M (Rast) *	—	—	—	—	—	—	271
n_D^{20}	1·4562	1·4569	1·4576	1·4577	1·4584	1·4591	1·4596
Reduction of Fehling's soln. and ammoniacal $AgNO_3$							Slight

At 55°.

Time (hours)	4	8	12	16	20	24	
Peroxide value	0·078	0·092	0·127	0·160	0·185	0·235	
I value	75·6	74·7	74·6	73·7	74·3	72·5	
Sap. value	167	169	170	172	173	174	
M (Rast) *	—	—	—	—	—	348	
n_D^{20}	1·4560	1·4562	1·4564	1·4567	1·4568	1·4571	
Reduction of Fehling's soln. and ammoniacal $AgNO_3$							None

* Calc. for $C_{22}H_{42}O_2$: *M*, 338·6.

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15. *Some Analogues of Chloramphenicol.*

By J. N. Ashley and M. Davis.

2-Dichloroacetamido-propane-1 : 3-diol, -2-hydroxymethylbutanol, -2-phenylpropane-1 : 3-diol, -2-*p*-nitrophenylpropane-1 : 3-diol, -4-methylpentane-1 : 3-diol, -3-*cyclo*hexylpropane-1 : 3-diol, and -3-*cyclo*hexylpropan-1-ol have been synthesised by standard methods. The products have no significant bactericidal or virucidal activity.

Since the publications of Rebstock, Crooks, Controulis, and Bartz (*J. Amer. Chem. Soc.*, 1949, **71**, 2458) and of Controulis, Rebstock, and Crooks (*ibid.*, p. 2463) dealing with the structure and synthesis of chloramphenicol, several analogues of this antibiotic have been synthesised. The products differ from chloramphenicol mainly in replacement of the *p*-nitro-group by other groups or elements in the *para* or other positions or in replacement of the dichloroacetyl by other acyl groups. Either of these changes is sufficient, in general, to decrease the biological activity.

We have prepared some simple analogues of chloramphenicol, in some of which the phenyl group was replaced by an alkyl group or by a reduced ring. These compounds were either inactive, or had no significant activity in *vitro* against *Staph. aureus*, *B. coli*, and *Ps. pyocyanea*, and *in vivo* against influenza, mouse pneumonitis, and *B. typhi*.

2-Dichloroacetamidopropane-1 : 3-diol, 2-dichloroacetamido-2-hydroxymethylbutanol and 2-dichloroacetamido-2-phenylpropane-1 : 3-diol were readily obtained by catalytically reducing the corresponding 2-nitropropane-1 : 3-diols and heating the resulting amino-diols with methyl dichloroacetate (Controulis, *et al.*, *loc. cit.*). In addition, 2-dichloroacetamido-2-*p*-nitrophenylpropane-1 : 3-diol, a positional isomer of chloramphenicol, was prepared by a route essentially identical with that subsequently described by Ruoff and Miller (*J. Amer. Chem. Soc.*, 1950, **72**, 1417). Since its properties agree with those given by the latter authors, only those intermediates not reported by the American workers are described in the present communication.

None of these four compounds possesses an asymmetric carbon atom; two other analogues which were prepared, 2-dichloroacetamido-4-methylpentane-1 : 3-diol and 2-dichloroacetamido-3-*cyclo*hexylpropane-1 : 3-diol, possess two asymmetric carbon atoms and should exist in both the *threo*- and the *erythro*-configuration. In both cases, however, only one of the two possible racemates was ever isolated.

The preparation of 4-methyl-2-nitropentane-1 : 3-diol was first attempted by condensation of 3-methyl-1-nitrobutan-2-ol (Schmidt, Ascherl, and Mayer, *Ber.*, 1925, **58**, *B*, 2430) with aqueous formaldehyde in the presence of potassium carbonate or sodium hydroxide, following Sprang and Degering's technique (*J. Amer. Chem. Soc.*, 1942, **64**, 1735; 1943, **65**, 628). This led to an oily mixture from which the required compound could not be isolated. The required nitro-diol was eventually obtained by condensation of *iso*butaldehyde with 2-nitroethanol in the presence of methanolic sodium methoxide, whereupon the sodium salt separated in reasonably good yield. Liberation of the diol was best effected with salicylic acid in ether (Schmidt and Wilkendorf, *Ber.*, 1919, **52**, 389). It formed an oil (possibly a mixture of racemates) which failed to crystallise when kept, but which was readily reduced catalytically over Raney nickel to the corresponding base, characterised as the crystalline triacetyl derivative. Condensation with methyl dichloroacetate then yielded the required 2-dichloroacetamido-4-methylpentane-1 : 3-diol. Reduction of the nitro-diol over platinum oxide or palladium oxide in acetic acid was slow and incomplete and the product contained a large proportion of non-basic material.

Small amounts of 2-acetamido-1 : 3-diacetoxypropane and 2-dichloroacetamidopropane-1 : 3-diol were invariably isolated during the preparation of the above compounds; they probably arose from some 2-nitropropane-1 : 3-diol present in the 2-nitroethanol used.

The condensation of *cyclo*hexanaldehyde with 2-nitroethanol was similarly effected in the presence of sodium methoxide, and 3-*cyclo*hexyl-2-nitropropane-1 : 3-diol was liberated from the resulting sodium salt by acetic acid and reduced over platinum oxide. 2-Amino-3-

*cyclo*hexylpropane-1 : 3-diol was isolated as its hydrochloride and converted into the dichloroacetamide. It was possible to assign to the product the *erythro*-configuration, since it differed from the authentic *threo*-compound obtained by Dr. B. J. Heywood (unpublished observation) by complete catalytic reduction of ±-*threo*-2-nitro-3-phenylpropane-1 : 3-diol.

When the sodium salt of the 3-*cyclo*hexyl-2-nitropropane-1 : 3-diol was dissolved in cold dilute hydrochloric acid, dehydration occurred (cf. Emerson, *Chem. Reviews*, **1949**, **45**, 368; Hass and Riley, *ibid.*, **1943**, **32**, 410); the resulting unsaturated nitro-alcohol was reduced by either lithium aluminium hydride (Gilsdorf and Nord, *J. Org. Chem.*, **1950**, **15**, 807) or catalytically over platinum oxide or Raney nickel, yielding 2-amino-3-*cyclo*hexylpropan-1-ol, which was isolated as the hydrochloride. Reaction of the base with methyl dichloroacetate then afforded the expected 2-dichloroacetamido-3-*cyclo*hexylpropan-1-ol.

An attempt to prepare 2-dichloroacetamido-2-1′-hydroxy*cyclo*hexylethanol a lower homologue of 2-dichloroacetamido-3-*cyclo*hexylpropane-1 : 3-diol was unsuccessful. Condensation of *cyclo*hexanone with nitromethane according to Frazer and Kon's method (*J.*, 1943, 604) proceeded readily and the difficulties described by Nightingale, Erickson, and Knight (*J. Org. Chem.*, **1950**, **15**, 782; cf. Hass and Riley, *loc. cit.*, p. 384; Grob and Tscharner, *Helv. Chim. Acta*, **1950**, **33**, 1070; Kon, *J.*, **1951**, 843) were not encountered, but further condensation of 1-nitromethyl*cyclo*hexanol with aqueous formaldehyde in the presence of potassium carbonate or sodium hydroxide, or with paraformaldehyde in the presence of methanolic potassium hydroxide, could not be effected. In addition, *cyclo*hexanone failed to react with 2-nitroethanol in the presence or sodium methoxide.

The preparation of some other less closely-related dichloroacetamides was also investigated. Reduction of *cyclo*hexanone cyanohydrin over a platinum oxide catalyst in acetic acid yielded a mixture of 1-aminomethyl*cyclo*hexanol and bis*cyclo*hexylmethylamine (both of which were converted into the respective *N*-dichloroacetyl derivatives), although Goldberg and Kirchensteiner (*Helv. Chim. Acta*, **1943**, **26**, 288) found that this method gave a mixture of 1-aminomethyl*cyclo*hexanol and di-(1-hydroxy*cyclo*hexylmethyl)-amine. Tchoubar (*Bull. Soc. chim.*, **1949**, **16**, 160) obtained the primary amine by carrying out the reduction in the presence of carbon disulphide, hydrochloric acid, or hydrogen cyanide. Bis*cyclo*hexylmethylamine has recently been obtained by Nightingale, Erickson, and Knight (*loc. cit.*) by high pressure reduction of 1-nitromethyl*cyclo*hex-1-ene over Raney nickel.

Experimental

M. p.s are uncorrected. Activated alumina (Peter Spence type H) was used for chromatographic work.

2-Dichloroacetamidopropane-1 : 3-diol.—2-Nitropropane-1 : 3-diol (Otter, *Rec. Trav. chim.*, **1938**, **57**, 13) (3·3 g.) in methanol (40 ml.) was reduced over Raney nickel at atmospheric temperature and pressure. When the uptake of hydrogen ceased (after 3 hours), the solution was filtered and evaporated, and the residue heated on the steam-bath with methyl dichloroacetate (7 g.) for 2 hours. After removal of excess of ester *in vacuo*, the residue was crystallised from acetone–benzene. Recrystallisation of the product (2·35 g.; m. p. 132—134°) from the same solvents gave *2-dichloroacetamidopropane-1 : 3-diol* as glistening plates, m. p. 144—146° (Found: N, 7·1; Cl, 34·8. $C_5H_9O_3NCl_2$ requires N, 6·9; Cl, 35·2%). The 1 : 2 : 3-triacetyl derivative (see below) formed needles, m. p. 79—80°.

2-Dichloroacetamido-2-hydroxymethylbutanol.—2-Amino-2-hydroxymethylbutanol (Vanderbilt and Hass, *Ind. Eng. Chem.*, **1940**, **32**, 34) (5·5 g.) and methyl dichloroacetate (14·2 g.) were heated on the steam-bath for 18 hours. After removal of excess of ester *in vacuo*, the residue was dissolved in benzene and chromatographed. Elution with benzene and benzene–chloroform, followed by crystallisation from acetone–light petroleum, gave *2-dichloroacetamido-2-hydroxymethylbutanol* (1·35 g.; this does not represent the maximum yield) as prisms, m. p. 108° (Found: N, 6·0; Cl, 30·7. $C_7H_{13}O_3NCl_2$ requires N, 6·1; Cl, 30·85%).

2-Amino-2-phenylpropane-1 : 3-diol.—2-Nitro-2-phenylpropane-1 : 3-diol was reduced over Raney nickel in methanol at atmospheric temperature and pressure. The product (66%; m. p. 116—117°) was crystallised from ethyl acetate; it then had m. p. 117—118° (Found: C, 64·8; H, 7·8; N, 8·2. Calc. for $C_9H_{13}O_2N$: C, 64·7; H, 7·8; N, 8·4%) (Ruoff and Miller,

loc. cit., give 50% yield; m. p. 116—118·5°). The *hydrochloride* formed plates, m. p. 181—182°, from methanol–ether (Found: N, 7·0; Cl, 17·0. $C_9H_{13}O_2N$,HCl requires N, 6·9; Cl, 17·9%).

2-*Dichloroacetamido*-2-*phenylpropane*-1 : 3-*diol*.—2-Amino-2-phenylpropane-1 : 3-diol (1·3 g.) and methyl dichloroacetate (5·2 g.) were heated on the steam-bath for 4·5 hours. After removal of excess of ester, the residue was dissolved in ethyl acetate, washed free from unchanged base, evaporated, and chromatographed in benzene. Elution with benzene and ether gave 2-*dichloroacetamido*-2-*phenylpropane*-1 : 3-*diol* (0·8 g) as prisms, m. p. 105—106°, from ethyl acetate–light petroleum (Found: N, 5·1; Cl, 25·6. $C_{11}H_{13}O_3NCl_2$ requires N, 5·0; Cl, 25·5%).

2-*Acetamido*-2-p-*nitrophenylpropane*-1 : 3-*diol*.—The ethyl acetate mother-liquors from the crystallisation of 2-amino-2-*p*-nitrophenylpropane-1 : 3-diol during 1—2 weeks deposited a small quantity of 2-*acetamido*-2-p-*nitrophenylpropane*-1 : 3-*diol* as long prisms; m. p. 178° (Found: C, 51·7; H, 5·6. $C_{11}H_{14}O_5N_2$ requires C, 51·9; H, 5·6%).

4-*Methyl*-2-*nitropentane*-1 : 3-*diol*.—A solution of sodium methoxide [from sodium (5·7 g.) and dry methanol (100 ml.)] was added during 40 minutes to a stirred solution of freshly-distilled *iso*butaldehyde (18 g.) and 2-nitroethanol (22·5 g.) in dry methanol (50 ml.). The solution was cooled, and the *sodium* salt (24·4 g.; 53%) which separated was filtered off and washed with a little cold methanol and with ether. A sample was purified for analysis by precipitation of its aqueous solution with acetone (Found: N, 7·5; Na, 13·2. $C_6H_{12}O_4NNa$ requires N, 7·6; Na, 12·4%). The finely powdered sodium salt (11·3 g.) was boiled with salicylic acid (8 g.) in dry ether (150 ml.) for 2·5 hours. The solution was filtered and evaporated, and the residue dried over sulphuric acid *in vacuo*. The yield was 7·5 g. (75%).

2-*Amino*-4-*methylpentane*-1 : 3-*diol*.—The foregoing nitro-diol (7·5 g.) in methanol (100 ml.) was reduced over Raney nickel, the crude 2-amino-4-methylpentane-1 : 3-diol (4·8 g.) being obtained as an oil. A small amount of the base was dissolved in acetic anhydride–pyridine and kept overnight. Excess of reagents was removed *in vacuo* and the residue chromatographed in benzene–light petroleum. Elution with benzene and ether gave 2-*acetamido*-1 : 3-*diacetoxy*-4-*methylpentane* as plates, m. p. 100—102°, from ether–light petroleum (Found: C, 55·85; H, 8·2. $C_{12}H_{21}O_5N$ requires C, 55·6; H, 8·2%). Further elution of the column with acetone gave a small quantity of 2-*acetamido*-1 : 3-*diacetoxypropane* as needles, m. p. 79—80°, from ether (Found: C, 50·0; H, 7·1. $C_9H_{15}O_5N$ requires C, 49·8; H, 7·0%).

2-*Dichloroacetamido*-4-*methylpentane*-1 : 3-*diol*.—The base obtained by reduction of 4-methyl-2-nitropentane-1 : 3-diol (1·2 g.) was heated for 2 hours on the steam-bath with a large excess of methyl dichloroacetate. After removal of excess of ester *in vacuo*, the residue was chromatographed in benzene. Elution with benzene and ether gave 2-*dichloroacetamido*-4-*methylpentane*-1 : 3-*diol* (0·4 g.; m. p. 137°), which separated in plates, m. p. 140—141°, from acetone–light petroleum (Found: C, 39·6; H, 6·3; N, 6·3; Cl, 28·7. $C_9H_{15}O_3NCl_2$ requires C, 39·3; H, 6·2; N, 5·7; Cl, 29·1%). In a similar experiment in which the base from nitro-diol sodium salt (10·85 g.) was heated for 16 hours at 100° with methyl dichloroacetate (16·8 g.), there were obtained, after chromatography, 2-dichloroacetamido-4-methylpentane-1 : 3-diol (1·15 g.) and 2-dichloroacetamidopropane-1 : 3-diol (0·55 g.) (Found: N, 7·0; Cl, 35·1%), m. p. and mixed m. p. 142—144°.

3-cyclo*Hexyl*-2-*nitropropane*-1 : 3-*diol*.—A solution of sodium methoxide [from sodium (5 g.) and methanol (150 ml.)] was slowly added to a stirred solution of *cyclo*hexanaldehyde (Wood and Comley, *J. Soc. Chem. Ind.*, 1923, **42**, 431T; cf. Heilbron, Jones Richardson, and Sondheimer, *J.*, 1949, 737) (24·3 g.) and 2-nitroethanol (19·8 g.) in methanol (50 ml.). A transient orange colour was observed at the first addition. After 1—2 hours, the sodium salt (34 g.; 70%) was filtered off and washed with a small amount of cold methanol and with ether. For analysis a sample was precipitated from its aqueous solution with acetone (Found: N, 5·9; Na, 10·6. $C_9H_{16}O_4NNa$ requires N, 6·2; Na, 10·2%).

2-*Amino*-3-cyclo*hexylpropane*-1 : 3-*diol*.—The above sodium salt (14·6 g.) in acetic acid (200 ml.) was reduced over Adams's platinum oxide (1 g.) at atmospheric temperature and pressure. When the uptake of hydrogen ceased, the solution was filtered and treated with fresh catalyst. After 4 hours, 4 g. of catalyst had been used and the uptake was 3·15 l. (theory 5·2 l.). The solution was then filtered and evaporated *in vacuo*, and the residue dissolved in 2N-hydrochloric acid (200 ml.) and extracted thrice with ether. Evaporation of the acid solution and crystallisation of the residue from methanol–ether gave the crude hydrochloride (3·4 g.; m. p. 120—140°). By recrystallisation from methanol–ether and methanol–benzene, 2-*amino*-3-cyclo*hexylpropane*-1 : 3-*diol hydrochloride* was obtained as long prisms, m. p. 240—242° (softens 172°) (Found: C, 51·4; H, 9·9; N, 7·1. $C_9H_{19}O_2N$,HCl requires C, 51·5; H, 9·6; N, 6·7%).

2-*Dichloroacetamido*-3-cyclo*hexylpropane*-1 : 3-*diol*.—The crude base hydrochloride (3·2 g.) was dissolved in methanolic potassium hydroxide (2·46N; 6·21 ml.) and the solution evaporated to dryness. The residue was heated on the steam-bath with a large excess of methyl dichloroacetate for 3·25 hours, the solution evaporated *in vacuo*, and the residue dissolved in benzene containing a small amount of acetone and chromatographed. Elution with benzene and ether gave a trace of 2-dichloroacetamido-3-*cyclo*hexylpropan-1-ol (60 mg.), m. p. 114° (see below). Elution with ether–acetone and acetone gave 2-*dichloroacetamido*-3-cyclo*hexylpropane*-1 : 3-*diol* (1·03 g.), which separated from acetone–benzene as plates, m. p. 162—163° (Found : C, 46·5; H, 6·7. $C_{11}H_{19}O_3NCl_2$ requires C, 46·5; H, 6·7%).

2-*Amino*-3-cyclo*hexylpropan*-1-*ol*.—(*a*) A solution of the nitro-diol sodium salt (4·5 g.) in 2N-hydrochloric acid was extracted thrice with ether. The washed and dried extracts were slowly added to a stirred solution of lithium aluminium hydride (1·52 g.) in dry ether. After being refluxed for 2 hours, the solution was decomposed with water, treated with sodium hydroxide, and repeatedly extracted with chloroform and ethyl acetate. The washed, dried extracts were evaporated, the residual oil (1·5 g.) dissolved in 2N-hydrochloric acid and the solution extracted with ether and then evaporated *in vacuo*. 2-*Amino*-3-cyclo*hexylpropan*-1-*ol hydrochloride* separated from methanol on the addition of acetone and benzene as glistening plates, m. p. 195° (Found : N, 7·1; Cl, 18·2. $C_9H_{19}ON,HCl$ requires N, 7·2; Cl, 18·3%).

(*b*) The sodium salt (6·4 g.) was acidified as described above, and the product reduced over Raney nickel in ethanol at 20° and 5 atm. pressure. The basic portion of the product gave the same hydrochloride (1·1 g.), m. p. 196°.

(*c*) The nitro-alcohol from the sodium salt (17 g.) was reduced over Adams's platinum oxide in acetic acid. The basic portion of the product yielded the above hydrochloride (8·3 g.) m. p. 192—193°.

2-*Dichloroacetamido*-3-cyclo*hexylpropan*-1-*ol*.—The base hydrochloride [2·25 g. from (*c*) above] was dissolved in 0·815N-methanolic potassium hydroxide (13·45 ml.) and the solution evaporated; the residue was heated with methyl dichloroacetate (10 g.) for 2·5 hours on the steam-bath and excess of ester distilled off. The crude product was chromatographed in benzene. Elution with benzene and ether gave 2-*dichloroacetamido*-3-cyclo*hexylpropan*-1-*ol* (1·8 g.) as leaflets, m. p. 114°, from acetone–light petroleum (Found : C, 49·5; H, 7·2. $C_{11}H_{19}O_2NCl_2$ requires C, 49·6; H, 7·2%). The *acetate*, obtained by treatment of the propanol with acetic anhydride–pyridine overnight, formed long narrow prisms, m. p. 80—81°, from light petroleum (Found : C, 50·6; H, 7·0; N, 4·5. $C_{13}H_{21}O_3NCl_2$ requires C, 50·3; H, 6·8; N, 4·5%). Further elution of the column with acetone gave a trace of 2-dichloroacetamido-3-*cyclo*hexylpropane-1 : 3-diol (60 mg.), m. p. 160—162°.

Reduction of cyclo*Hexanone Cyanohydrin*.—*cyclo*Hexanone cyanohydrin (Frank, Berry, and Shotwell, *J. Amer. Chem. Soc.*, 1949, **71**, 3891) (47·5 g.) in acetic acid (150 ml.) was reduced over Adams's platinum oxide (5 g.) at atmospheric temperature and pressure. Hydrogen uptake was 17·4 l. in 36 hours. The solution was evaporated *in vacuo*, and the residue treated with 20% sodium hydroxide and extracted five times with ether (total 500 ml.). The washed and dried extracts were evaporated on the steam-bath and then distilled at 0·8—1 mm., fractions being collected at (1) <100° (11·7 g.), (2) 100—119° (3·25 g.), and (3) 120—144° (10·4 g.).

1-*Dichloroacetamidomethyl*cyclo*hexan*-1-*ol*. (*a*) Fraction (1) (2·8 g.) was dissolved in benzene (20 ml.), treated with dichloroacetyl chloride (1 ml.), and heated under reflux for 1 hour on the steam-bath. The cooled solution was diluted with ether, filtered from 1-aminomethyl*cyclo*hexanol hydrochloride, m. p. 209—210° (Goldberg and Kirchensteiner, *loc. cit.*, give m. p. 210—212°; Tchoubar, *loc. cit.*, gives m. p. 190°), washed with dilute hydrochloric acid, sodium hydrogen carbonate solution and water, and dried, concentrated, and cooled. 1-*Dichloroacetamidomethyl*cyclo*hexan*-1-*ol* (1·4 g.) crystallised from benzene–light petroleum in plates, m. p. 122—124° (Found : N, 5·85; Cl, 29·5. $C_9H_{15}O_2NCl_2$ requires N, 5·8; Cl, 29·6%).

(*b*) The same compound, m. p. and mixed m. p. 122—124°, was the only crystalline product isolated when 1-nitromethyl*cyclo*hexanol (Frazer and Kon, *loc. cit.*) in alcohol (25 ml.) and 5·8N-sodium hydroxide (1 ml.) was treated with 40% formaldehyde (7·5 ml.), and the solution kept for 6 days, neutralised with 2N-hydrochloric acid (2·9 ml.), concentrated at 25 mm. and then at 0·8 mm. (bath temp. 55°), reduced over Raney nickel in methanol and finally heated on the steam-bath with methyl dichloroacetate.

N-*Dichloroacetylbis*cyclo*hexylmethylamine*. Fraction (3) (3·55 g.) and dichloroacetyl chloride (1·5 ml.) in dry benzene (30 ml.) were heated under reflux for one hour on the steam-bath, cooled, diluted with ether, and filtered from bis*cyclo*hexylmethylamine hydrochloride (0·6 g.), m. p. 271—272° (decomp.) (Found : N, 5·7; Cl, 14·1. Calc. for $C_{14}H_{27}N,HCl$: N, 5·7; Cl,

14·5%) (Nightingale, Erickson, and Knight, *J. Org. Chem.*, 1950, **15**, 782, give m. p. 264—265°). The ethereal solution was washed with dilute hydrochloric acid, sodium hydrogen carbonate solution, and water, dried, concentrated, and treated with light petroleum, to give N-*dichloroacetylbis*cyclo*hexylmethylamine* (2·1 g.), prisms from acetone–light petroleum, m. p. 168—170° (Found: N, 4·4; Cl, 22·1. $C_{16}H_{27}ONCl_2$ requires N, 4·4; Cl, 22·2%).

The authors thank Dr. A. J. Ewins, F.R.S., for his interest in this work, Mr. W. A. Freeman of the Biological Division for the antibacterial and virucidal tests, Mr. S. Bance, B.Sc., A.R.I.C., for the semi-microanalyses, and the Directors of May and Baker Ltd. for permission to publish these results.

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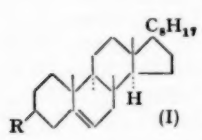
16. *Some Basic Steroid Derivatives.*

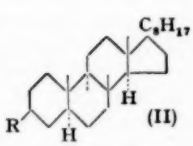
By DOUGLAS P. DODGSON and ROBERT D. HAWORTH.

As the amœbicidal properties of conessine might have been associated with the steroidal structure of the alkaloid, a number of basic steroid derivatives have been prepared, but none of their pharmacological activities appears to be greater than that of conessine.

MANY compounds, chiefly tertiary diamines and certain alkaloids, *e.g.*, emetine, have been used or suggested as amœbicidal agents, but as a result of the lack of a suitable *in vivo* technique, many of the compounds previously tested gave high activity *in vitro* which did not necessarily correspond to their activity *in vivo*. A more suitable method for a preliminary evaluation of activity *in vivo* has however recently been developed (Jones, *Ann. Trop. Med. Parasit.*, 1946, **40**, 130; *Brit. J. Pharmacol.*, 1947, **2**, 217). It has long been known that extract of *Holarrhena antidysenterica* is valuable in the treatment of amœbic dysentery, and the activity of conessine, the major alkaloidal constituent of the extract, has been confirmed by the new technique referred to above. In view of the established steroid nature of conessine it was decided to prepare a number of basic steroid derivatives and the following compounds, none of which are sufficiently active to be effective in low doses, have been tested as amœbicidal agents: 3β-dimethylaminocholest-5-ene (I; R = NMe_2), 3β-dimethylaminocholestane (II; R = NMe_2), 3β-2′-diethylaminoethylaminocholest-5-ene (I; R = $NH{\cdot}CH_2{\cdot}CH_2{\cdot}NEt_2$), 3β-(*N*-2-diethylaminoethyl-*N*-methylamino)cholest-5-ene (I; R = $NMe{\cdot}CH_2{\cdot}CH_2{\cdot}NEt_2$), 23-amino-3α : 7α : 12α-trihydroxynorcholane (III; R = OH, R′ = NH_2), 23-dimethylamino-3α : 7α : 12α-trihydroxynorcholane (III; R = OH, R′ = NMe_2), methyl 3ξ-dimethylamino-7α : 12α-dihydroxycholanate* (III; R = NMe_2, R′ = CO_2Me), 3ξ : 23-bisdimethylamino-7α : 12α-dihydroxynorcholane (III; R = R′ = NMe_2), 3ξ : 17ξ-bisdimethylaminoandrostane (IV).



(I)



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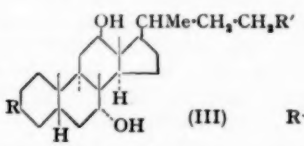

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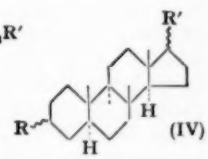


(IV)

3β-Dimethylaminocholest-5-ene (I; R = NMe_2) was prepared by heating cholesteryl chloride with methanolic dimethylamine at 180°, with dimethylamine hydriodide as catalyst, and reduction with hydrogen in presence of palladium–charcoal in acetic acid solution gave 3β-dimethylaminocholestane (II; R = NMe_2). This base was also obtained by reducing 3-oximinocholestane (Fujii and Matsukawa, *J. Pharm. Soc. Japan*, 1936, **56**, 642) with sodium and amyl alcohol to 3β-aminocholestane, which was methylated by

* The symbol ξ in names, and the wavy bond in formulae, signify linkages of uncertain configuration (cf. *Chem. and Ind.*, 1951, June 23rd, p. SN7).

heating it with formaldehyde and formic acid. The intermediate 3β-aminocholestane (II; R = NH_2) obtained by reducing the oximino-compound was the major constituent of a mixture which was acetylated and separated into two acetamido-derivatives melting at 245—246° and 213° respectively; the former, on hydrolysis with acids gave the 3β-amino-derivative which had m. p. 106—120°. This amine, probably a component of the mixture isolated by Windaus and Adamla (*Ber.*, 1911, **44**, 3051), gave, in spite of the wide melting range, high yields of pure derivatives on acetylation or methylation.

3β-2′-Diethylaminoethylaminocholest-5-ene (I; R = $NH{\cdot}CH_2{\cdot}CH_2{\cdot}NEt_2$), prepared by the action of 2-diethylaminoethylamine on cholesteryl chloride or preferably on the toluene-*p*-sulphonate, partly decomposed during attempted distillation to cholest-3 : 5-diene and 2-diethylaminoethylamine. It gave a sparingly soluble hydrochloride and a characteristic dipicrate from either of which the base was regenerated in a crystalline form, m. p. 64°. Methylation of the base with formaldehyde and formic acid yielded 3β-(*N*-2-diethylaminoethyl-*N*-methylamino)cholest-5-ene (I; R = $NMe{\cdot}CH_2{\cdot}CH_2{\cdot}NEt_2$), as an oil which was characterised as the dihydrochloride, m. p. 280°, and the dipicrate, m. p. 243—245°.

Whilst a detailed study of the stereochemistry of these basic cholestenes and cholestanes has not been possible, it is suggested that the compounds have the 3β-configuration in agreement with Shoppee's conclusions (*J.*, 1946, 1147) concerning replacement reaction at $C_{(3)}$ in Δ^5-steroids. 3β-Aminocholestane like cholestan-3β-ol gives a digitonin complex quite readily and, although this reaction is not understood, the analogy is attractive; it is interesting in this connection to record that 3β-acetamidocholestane and 3β-dimethylaminocholestane do not give adducts with digitonin. The lævorotations observed with the 3β-dimethylamino-, 3β-2′-diethylaminoethylamino- and 3β-(*N*-2′-diethylaminoethyl-*N*-methylamino)cholest-5-enes are consistent with the lævorotation of cholesterol, and the reduction of the first compound to the dextrorotatory 3β-dimethylaminocholestane is not only reminiscent of the change in optical properties which accompanies the reduction of cholesterol to cholestan-3-ol, but the molecular rotation difference of −226° is in excellent quantitative agreement.

23-Dimethylamino-3α : 7α : 12α-trihydroxynorcholane (III; R = OH, R′ = NMe_2) was prepared by methylating with formaldehyde and formic acid the corresponding 23-amino-compound (III; R = OH, R′ = NH_2) obtained from cholic azide (Caldwell, *J. Amer. Chem. Soc.*, 1938, **60**, 991). Oppenauer oxidation with aluminium *tert.*-butoxide gave a dihydroxy-keto-derivative which is considered to be 23-dimethylamino-7α : 12α-dihydroxy-3-ketonorcholane by analogy with the preferential attack of the 3-hydroxy-group during similar oxidation of methyl cholate (Kuwada and Norimo, *Bull. Chem. Soc. Japan*, 1942, **17**, 147). The conversion of this keto tertiary amine into 3ξ : 23-bisdimethylamino-7α : 12α-dihydroxynorcholane (III; R = R′ = NMe_2) by oximation of the 3-keto-group and subsequent reduction and methylation was successful, although the oximation proved difficult in practice and only a few milligrams of the dihydrochloride of the amine were obtained by this method; an alternative procedure gave improved yields. 7α : 12α-Dihydroxy-3-ketocholanic acid was oximated and reduced to 3ξ-amino-7α : 12α-dihydroxycholanic acid as described by Jones, Smith, and Webb (*J.*, 1949, 2164). The primary amine was then methylated with formaldehyde, to methyl 3ξ-dimethylamino-7α : 12α-dihydroxycholanate, which was converted *via* the hydrazide and azide into 23-amino-3ξ-dimethylamino-7α : 12α-dihydroxynorcholane (III; R = NMe_2, R′ = NH_2) which was isolated as the dihydrochloride. Methylation with formaldehyde gave 3ξ : 23-bisdimethylamino-7α : 12α-dihydroxynorcholane (III; R = R′ = NMe_2) which was also isolated as the dihydrochloride, identical with the dihydrochloride prepared as outlined above.

3ξ : 17ξ-Bisdimethylaminoandrostane (IV; R = R′ = NMe_2) was obtained from 3 : 17-bishydroxyaminoandrostane (Ruzicka, Meister, and Prelog, *Helv. Chim. Acta*, 1947, **30**, 867) by reduction to the 3ξ : 17ξ-diamine (IV; R = R′ = NH_2) which was characterised as its ditoluene-*p*-sulphonate and as 3ξ : 17ξ-diacetamidoandrostane (IV; R = R′ = NHAc), and when methylated with formaldehyde in the usual manner gave 3ξ : 17ξ-bisdimethylaminoandrostane (IV; R = R′ = NMe_2), m. p. 101—102°.

Experimental

3β-*Dimethylaminocholest-5-ene* (I; R = NMe_2).—Cholesteryl chloride was prepared in 80% yield by the method of Daughenbaugh and Allison (*J. Amer. Chem. Soc.*, 1929, **51**, 3665) but with light petroleum (b. p. 40—60°) instead of pyridine as solvent. During preliminary experiments it was found that the action of warm thionyl chloride on cholesterol gave a compound, $C_{27}H_{46}Cl_2$, m. p. 118°, probably identical with 3 : 5-dichlorocholestane obtained previously by Mauthner (*Monatsh.*, 1906, **27**, 305, 421) by the action of hydrogen chloride on cholesterol or cholesteryl chloride.

Cholesteryl chloride (3 g.), 50% methanolic dimethylamine (20 c.c.), and dimethylamine hydriodide (0·6 g.) were heated for 18 hours at 180°. Hydrogen chloride was passed into a washed and dried ethereal extract of the product, and the gelatinous water-insoluble hydrochloride was collected, crystallised from methanol, and basified. **3β-*Dimethylaminocholest-5-ene*** (I; R = NMe_2) separated from ether in long prisms (0·3 g.), m. p. 151°, $[\alpha]_D^{20}$ −31·5° ± 1·0° (*c*, 0·5 in chloroform) (Found: C, 84·2; H, 12·2. $C_{29}H_{51}N$ requires C, 84·2; H, 12·4%). 3β-Methoxycholest-5-ene, m. p. 83°, was isolated from the non-basic portion from the reaction, but attempts to improve the yields of the base (I; R = NMe_2) by using non-polar solvents or excess of dimethylamine as solvent failed and considerable quantities of cholesteryl chloride were recovered.*

3β-*Aminocholestane* (II; R = NH_2).—Cholesterol was converted into cholestan-3β-ol and cholestan-3-one as described in *Org. Synth.*, XVII, pp. 43, 45. 3-Hydroxyaminocholestane, prepared in ethanol, separated from ethyl acetate in colourless needles, m. p. 196°, $[\alpha]_D^{20}$ +38° (*c*, 0·25 in chloroform) (Found: C, 80·4; H, 12·0. Calc. for $C_{27}H_{47}ON$: C, 80·7; H, 11·8%). Fujii and Matsukawa (*loc. cit.*) give m. p. 187° (decomp.). The oxime (1 g.) in amyl alcohol (70 c.c.) was reduced by gradual addition of sodium (6 g.) during 2—3 hours to the boiling solution. The alcohol was washed with water and removed under reduced pressure, and the residue was taken up in ether. The hydrochlorides precipitated by hydrogen chloride were collected and basified, and the amine mixture, m. p. 90—120°, was recovered. Acetylation by boiling acetic anhydride (1 c.c.) in ether (10 c.c.) gave **3β-*acetamidocholestane***, which crystallised from alcohol in hexagonal plates, m. p. 245—246°, $[\alpha]_D^{20}$ +12° (*c*, 0·2 in chloroform) (Found, after drying for 8 hours at 100° at 0·5 mm.: C, 81·3; H, 11·8. $C_{29}H_{51}ON$ requires C, 81·0; H, 12·0%). The mother-liquors yielded an ***isomer***, which separated from ether in slender needles, m. p. 213° (Found, after drying for 8 hours at 100° at 0·001 mm.: C, 81·5; H, 11·6%), but the quantity available was too small for further examination. 3β-Acetamidocholestane (0·25 g.) was heated under reflux in ethanol (210 c.c.) and concentrated hydrochloric acid (140 c.c.) for 24 hours, then evaporated to dryness, and the basified product was isolated with ether. **3β-*Aminocholestane*** (II; R = NH_2) separated from ethyl alcohol in colourless needles, m. p. 106—120°, which adhere to solvent (Found, after drying at 100° and 0·001 mm.: C, 83·4; H, 12·5. $C_{27}H_{49}N$ requires C, 83·7; H, 12·7%) and readily give a precipitate with digitonin.

3β-*Dimethylaminocholestane* (II; R = NMe_2).—(*a*) 3β-Dimethylaminocholest-5-ene (0·25 g.) in acetic acid (3 c.c.) was rapidly reduced with hydrogen in presence of palladium–charcoal (0·4 g.), and the product was isolated with ether. (*b*) 3β-Aminocholestane (60 mg.; m. p. 90—120°) was heated for 3 hours on a water-bath with 90% formic acid (1·25 c.c.) and 40% formaldehyde (1 c.c.). Traces of unchanged primary amine were removed by addition of ethanol (1 c.c.), concentrated hydrochloric acid (0·5 c.c.), and sodium nitrite (20 mg.), and after 1 hour the product was taken up in ether and precipitated as the water-insoluble hydrochloride, and the base recovered. **3β-*Dimethylaminocholestane*** (II; R = NMe_2), prepared by either method, crystallised from ether in long prisms, m. p. 106°, $[\alpha]_D^{20}$ +23° ± 2·0° (*c*, 1·75 in chloroform) (Found, after drying at room temperature at 1 mm. for 3 hours: C, 83·9; H, 12·9. $C_{29}H_{53}N$ requires C, 83·8; H, 12·9%). This tertiary amine does not give a precipitate with digitonin.

3β-(2-*Diethylaminoethylamino*)*cholest-5-ene* (II; R = $NH{\cdot}CH_2{\cdot}CH_2{\cdot}NEt_2$).—(*a*) Cholesteryl chloride (2 g.), 2-diethylaminoethylamine (10·6 c.c.), and hydrogen iodide (1·2 g.) were heated at 145° for 18 hours (*b*) Cholesteryl toluene-*p*-sulphonate (Wallis, Fernholz, and Gephart, *J. Amer. Chem. Soc.*, 1937, **59**, 137) (20 g.), 2-diethylaminoethylamine (100 c.c.), and toluene-*p*-sulphonic acid (16 g.) were refluxed for 18 hours.

The mixture, from experiments (*a*) or (*b*), was basified and extracted with ether and evaporated, and unchanged 2-diethylaminoethylamine was removed under reduced pressure.

* Considerably increased yields of (I; R = NMe_2) have been obtained by the action of dimethylamine on cholesteryl toluene-*p*-sulphonate. These results will be reported later.

The residue was taken up in ether, and the hydrochloride, precipitated with dry hydrogen chloride, crystallised with difficulty from methanol–acetone in needles, m. p. 255—265°, and for subsequent stages was obtained sufficently pure by dissolving the crude salt in a minimum of methyl alcohol and reprecipitating it with ether. The crude *base*, obtained in yields of 25 and 70% by method (*a*) and (*b*) respectively, was recovered from the hydrochloride and crystallised from acetone in colourless needles, m. p. 64°, $[\alpha]_D^{20}$ −18° (*c*, 0·9 in ethanol) (Found : C, 81·6; H, 12·0. $C_{33}H_{60}N_2$ requres C, 81·8; H, 12·5%). The *dipicrate*, prepared in methyl alcohol, crystallised from acetic acid in yellow prisms, m. p. 224—226° (Found : C, 57·6; H, 6·8. $C_{33}H_{60}N_2,2C_6H_3O_7N_3$ requires C, 57·3; H, 7·1%), and gave the base (I; R = $NH{\cdot}CH_2{\cdot}CH_2{\cdot}NEt_2$), m. p. 64°, on treatment with sodium hydroxide.

3β-(N-2-*Diethylaminoethyl*-N-*methylamino*)*cholest*-5-*ene* (I; R = $NMe{\cdot}CH_2{\cdot}CH_2{\cdot}NEt_2$).—The base described above (1 g.) was heated on a water-bath for 2 hours with 90% formic acid (5 c.c.) and 40% formaldehyde (3 c.c.). After basification, the product was isolated with ether, and the *dihydrochloride* precipitated and collected; it crystallised from methyl alcohol in colourless needles, m. p. 280° (decomp.), $[\alpha]_D^{20}$ −12° (*c*, 0·5 in methyl alcohol) (Found : C, 70·9; H, 11·0. $C_{34}H_{62}N_2,2HCl$ requires C, 71·4; H, 11·3%). The *dipicrate* separated from acetic acid in yellow needles, m. p. 243—245° (decomp.) (Found : C, 58·0; H, 7·1. $C_{34}H_{62}N_2,2C_6H_3O_7N_3$ requires C, 57·7; H, 7·2%).

23-*Dimethylamino*-3α : 7α : 12α-*trihydroxynorcholane* (III; R′ = OH, R′ = NMe_2).—Methyl cholate (Reichstein and Grand, *Helv. Chim. Acta*, 1945, **28**, 344), b. p. 280° (bath-temp.)/0·001 mm., was converted *via* cholic hydrazide and azide (Bondi and Muller, *Z. physiol. Chem.*, 1906, **47**, 499) into 23-amino-3α : 7α : 12α-trihydroxynorcholane (Caldwell, *loc. cit.*), m. p. 185—186°, b. p. 260—270° (bath-temp.)/0·001 mm., $[\alpha]_D^{20}$ +50° (*c*, 0·5 in ethyl alcohol) (Found : C, 72·4; H, 10·8. Calc. for $C_{23}H_{41}O_3N$: C, 72·8; H, 10·8%). This primary amine (10 g.) was heated on a water-bath for 3 hours with 90% formic acid (12·5 c.c.) and 40% formaldehyde (10 c.c.). The product was evaporated under reduced pressure, dissolved in N-hydrochloric acid (400 c.c.), and treated with N-sodium nitrite (100 c.c.). After 1 hour the solution was rendered alkaline and the 23-*dimethylamino*-3α : 7α : 12α-*trihydroxynorcholane* (III; R = OH, R′ = NMe_2), isolated with ether, separated in cubic crystals, which melted about 130°, resolidified at 140°, and finally melted at 165—166°, $[\alpha]_D^{20}$ +46° (*c*, 0·5 in ethyl alcohol) (Found, after drying at 120°/2 mm. for 2 hours : C, 73·2; H, 10·7. $C_{25}H_{45}O_3N$ requires C, 73·7; H, 11·1%). This tertiary amine (III; R = OH, R′ = NMe_2) had b. p. 250—260°/0·005 mm. and gave a *hydrochloride* which crystallised from methyl alcohol–acetone in needles, m. p. 295—296° (Found : C, 67·4; H, 10·5. $C_{25}H_{46}O_3NCl$ requires C, 67·6; H, 10·4%).

23-*Dimethylamino*-7α : 12α-*dihydroxy*-3-*ketonorcholane*.—23-Dimethylamino-3α : 7α : 12α-trihydroxynorcholane (III; R = OH, R′ = NMe_2) (5 g.), aluminium *tert.*-butoxide (6·0 g.), benzene (150 c.c.), and acetone (50 c.c.) were refluxed for 24 hours. The mixture was cooled, decomposed with water, and extracted with ether, and the extract washed with N-hydrochloric acid (2 × 50 c.c.). The acid washings were made alkaline and the liberated base, isolated with ether, crystallised from acetone; 23-*dimethylamino*-7α : 12α-*dihydroxy*-3-*ketonorcholane* (3·9 g.) was obtained as colourless needles, m. p. 180—181° (Found : C, 73·6; H, 10·5. $C_{25}H_{43}O_3N$ requires C, 74·0; H, 10·7%). The *hydrochloride* separated from methyl alcohol–acetone in colourless needles, m. p. 271—273° (Found : C, 67·4; H, 10·2; Cl, 7·6. $C_{25}H_{44}O_3NCl$ requires C, 67·9; H, 10·1; Cl, 8·0%). The 2 : 4-*dinitrophenylhydrazone hydrochloride*, prepared in methyl alcohol containing concentrated hydrochloric acid (2 equivs.), separated from methyl alcohol in clusters of orange needles, m. p. 230—233° (Found : C, 59·5; H, 8·0. $C_{31}H_{48}O_6N_5Cl$ requires C, 59·8; H, 7·8%).

3ξ-*Amino*-23-*dimethylamino*-7α : 12α-*dihydroxynorcholane* (III; R = NH_2, R′ = NMe_2).—23-Dimethylamino-7α : 12α-dihydroxy-3-ketonorcholane (1 g.), sodium acetate (1 g.), and hydroxylamine hydrochloride (0·8 g.) were refluxed in ethyl alcohol for 24 hours. The mixture was neutralised, and the crude oxime, m. p. 125—150° (10 mg.), isolated with ether, was dissolved in amyl alcohol (35 c.c.) and reduced with sodium (2 g.). After addition of dilute hydrochloric acid, the mixture was evaporated to dryness under reduced pressure and the residue made alkaline and extracted with ether. 3ξ-Amino-23-dimethylamino-7α : 12α-dihydroxynorcholane was obtained as a pale yellow oil, b. p. 240—250° (bath-temp.)/0·001 mm., which gave a *dihydrochloride* as colourless needles, m. p. 319—323° (Found : Cl, 14·6. $C_{25}H_{48}O_2N_2Cl_2$ requires Cl, 14·8%), from methyl alcohol–acetone.

Methyl 3ξ-*Dimethylamino*-7α : 12α-*dihydroxycholanate* (III; R = NMe_2, R′ = CO_2Me).—3ξ-Amino-7α : 12α-dihydroxycholanic acid hydrochloride (5 g.), prepared as described by Jones,

Webb, and Smith (*loc. cit.*), was refluxed for 1½ hours with 90% formic acid (6 c.c.) and 40% formaldehyde (5 c.c.). Methyl alcoholic hydrogen chloride (10 c.c. of 1%) was repeatedly added to the solution which was evaporated until the formaldehyde was eliminated. A further 30 c.c. of the methyl-alcoholic hydrogen chloride was added and after 1 hour's refluxing dilute sodium hydrogen carbonate solution was added and the product isolated with ether. Concentration of the *moist* ethereal solution gave *methyl* 3ξ-*dimethylamino*-7α : 12α-*dihydroxycholanate* as long prisms (3·5 g.), m. p. 70—80° [Found, after drying at 100°/0·3 mm. (loss in wt., 4·6%) : C, 72·0; H, 10·8. $C_{27}H_{47}O_4N$ requires C, 72·2; H, 10·6%]. The *hydrochloride* separated from methyl alcohol–acetone in long feathery needles, m. p. >360° (Found, after drying for 12 hours at 100°/0·5 mm. over P_2O_5 : C, 66·5; H, 10·4; Cl, 7·4. $C_{27}H_{48}O_4NCl$ requires C, 66·7; H, 10·0; Cl, 7·3%).

23-*Amino*-3ξ-*dimethylamino*-7α : 12α-*dihydroxynorcholane* (III; R = NMe_2, R′ = NH_2).—Methyl 3ξ-dimethylamino-7α : 12α-dihydroxycholanate (2·5 g.) was heated on a water-bath for 48 hours with 90% hydrazine (12·5 c.c.) and ethyl alcohol (12·5 c.c.). The mixture was evaporated to dryness under reduced pressure and the residual hydrazide, dissolved in concentrated hydrochloric acid (2 c.c.) and ice (30 g.), was stirred at 0° for 1 hour with sodium nitrite (0·6 g.) and neutralised with sodium hydroxide. The crude azide was collected and decomposed by warming for ½ hour with acetic acid (12 c.c.) and water (6 c.c.). The base (III; R = NMe_2, R′ = NH_2), liberated by addition of sodium hydroxide and isolated with ether, was an oil, which yielded a *dihydrochloride* (1·1 g.), crystallising from methyl alcohol and acetone in needles, m. p. 326—330° (decomp.) (Found : Cl, 14·6. $C_{26}H_{48}O_2N_2Cl_2$ requires Cl, 14·8%).

3ξ : 23-*Bisdimethylamino*-7α : 12α-*dihydroxynorcholane* (III; R = R′ = NMe_2).—23-Amino-3ξ-dimethylamino-7α : 12α-dihydroxynorcholane dihydrochloride (0·8 g.) was heated for 2 hours with sodium hydrogen carbonate (0·32 g.), 90% formic acid (3 c.c.), and 40% formaldehyde (2·5 c.c.). The mixture was repeatedly evaporated to dryness under reduced pressure with 1% methyl-alcoholic hydrogen chloride and then rendered alkaline with sodium hydroxide, and the base, isolated with ether, yielded a dihydrochloride which crystallised from methyl alcohol–acetone in small hygroscopic prisms, m. p. 315—318° (Found : Cl, 13·9. $C_{27}H_{52}O_2N_2Cl_2$ requires Cl, 14·0%)

3ξ : 17ξ-*Diaminoandrostane* (III; R = R′ = NH_2).—3 : 17-Bishydroxyaminoandrostane (2 g.), m. p. 271—273° (from ethyl alcohol), $[\alpha]_D^{20}$ +60° (*c*, 0·15 in chloroform) (Found : C, 71·7; H, 9·1. Calc. for $C_{19}H_{30}O_2N_2$: C, 71·7; H, 9·4%), was reduced by sodium (11 g.) in boiling amyl alcohol (200 c.c.). After 2—3 hours the solution was acidified, the solvent removed under reduced pressure, the residue basified and extracted with ether and the crude dihydrochloride (2·1 g.) precipitated. The base, obtained from the dihydrochloride (0·2 g.), was taken up in ether (50 c.c.), dried, and mixed with acetic anhydride (0·3 c.c.). After 1 hour, 3ξ : 7ξ-*diacetamidoandrostane* (V; R = R′ = NHAc) separated and crystallised from aqueous ethyl alcohol in prisms, m. p. 300° (Found : C, 73·8; H, 10·1. $C_{23}H_{38}O_2N_2$ requires C, 73·8; H, 10·2%). A further sample of the crude base (IV; R = R′ = NH_2) was mixed in ether with toluene-*p*-sulphonic acid; the *ditoluene*-p-*sulphonate* crystallised from methyl alcohol–acetone in small needles, m. p. >360° (Found : C, 62·1; H, 7·6. $C_{19}H_{34}N_2.2C_7H_8O_3S$ requires C, 62·5; H, 7·9%).

3ξ : 17ξ-*Bisdimethylaminoandrostane* (IV; R = R′ = NMe_2).—Crude 3ξ : 17ξ-diaminoandrostane (1·5 g.) was heated for 2 hours with 90% formic acid (4 c.c.) and 40% formaldehyde (3 c.c.), and the mixture evaporated to dryness, basified, and extracted with ether. Removal of the ether gave an oil, b. p. 200°/0·001 mm., which separated from methyl alcohol in leaflets (1·0 g.), m. p. 101—102° (Found : C, 79·2; H, 12·0. $C_{23}H_{42}N_2$ requires C, 79·8; H, 12·2%). The *ditoluene*-p-*sulphonate*, prepared in ether, separated from methyl alcohol–acetone in short rods, m. p. 270—272°, $[\alpha]_D^{20}$ +8° (*c*, 0·2 in ethyl alcohol) (Found : C, 64·2; H, 8·5. $C_{23}H_{42}N_2.2C_7H_8O_3S$ requires C, 64·4; H, 8·4%). The *dihydrochloride* was very soluble in water and separated from methyl alcohol–acetone in prisms, m. p. 360°; it sublimes at 250°/0·5 mm. (Found : C, 66·2; H, 10·8. $C_{23}H_{42}N_2.2HCl$ requires C, 65·9; H, 10·5%).

Our thanks are offered to Imperial Chemical Industries Limited for a grant which has defrayed some of the expenses of the investigation, to their Pharmaceutical Section for the amœbicidal tests, to British Schering Ltd. for a gift of androsterone, and to the University for the award of a Henry Ellison Fellowship to one of us.

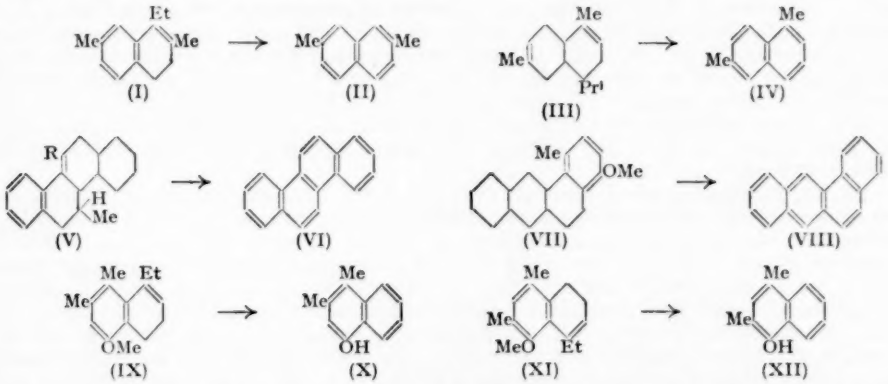
THE UNIVERSITY, SHEFFIELD, 10. [*Received, September 14th*, 1951.]

17. *The Elimination of Non-angular Alkyl Groups in Aromatisation Reactions. Part I.*

By Wesley Cocker, Brian E. Cross, and (Miss) Joan McCormick.

Dehydrogenation of a number of di- and tetra-hydronaphthalenes with palladised charcoal, selenium, and sulphur shows that in systems such as (XIII; R = Et, R′ = Me) the ethyl group is eliminated when selenium is the reagent.

Dehydrogenation of cyclic polyhydro-compounds by various reagents such as sulphur, selenium, palladium, and platinum has frequently been used as a means of assessing structure. In such reactions elimination of angular alkyl groups is the rule, but the value of the method for diagnostic purposes is generally based on the fact that, in simple systems at any rate, non-angular alkyl groups are unaffected by the process. Nevertheless, a number of instances are mentioned in the literature in which such alkyl groups are either eliminated or migrate during the aromatisation reaction. Thus, Ruzicka, Ehmann, and Mörgeli (*Helv. Chim. Acta*, 1933, **16**, 325) showed that 1-ethyl-3 : 4-dihydro-2 : 7-dimethylnaphthalene (I) is partly converted into 2 : 7-dimethylnaphthalene (II) by selenium at 300°. Cadinene (III) yields some 1 : 6-dimethylnaphthalene (IV) on being heated with selenium at 320° (Seidel, Müller, and Schinz, *ibid.*, 1944, **27**, 738), or with platinised charcoal at 305° (Linstead, Michaelis, and Thomas, *J.*, 1940, 1139). 1 : 2 : 3 : 4-Tetrahydro-1-*cyclo*pentylnaphthalene yields naphthalene when passed in a current of nitrogen over platinum at 310—315° (Denisenko and Naber, *Chem. Abs.*, 1940, **34**, 7283). Jones and Ramage (*J.*, 1938, 1853) showed that the perhydrochrysene (V; R = H or Me) yielded chrysene (VI) when heated with selenium for 72 hours at 280—300°, and Cook and Robinson (*J.*, 1938, 506) found that the decahydro-1 : 2-benzanthracene (VII) gave a small yield of 1 : 2-benzanthracene (VIII) at a similar temperature. Recently (*J.*, 1950, 1781) it was shown that 8-ethyl-5 : 6-dihydro-4-methoxy-1 : 2-dimethylnaphthalene (IX) yielded 3 : 4-dimethyl-1-naphthol (X) after demethylation and dehydrogenation with selenium at 330°. Similarly, 4-ethyl-1 : 2-dihydro-5-methoxy-6 : 8-dimethylnaphthalene (XI) yielded 2 : 4-dimethyl-1-naphthol (XII).



Examples are cited by Plattner (" Newer Methods of Preparative Organic Chemistry," New York, 1948, p. 21) in which migration of groups or change in carbon skeleton takes place during dehydrogenation, but the number of recorded cases where a non-angular alkyl group is eliminated from simple systems such as those mentioned, is relatively few.

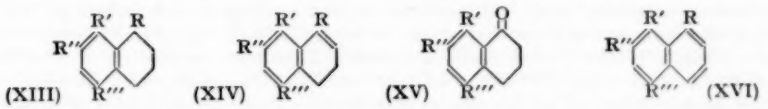
Further work is now described, which had as its objective the elucidation of factors which might be responsible for the loss of alkyl groups. Such factors as the nature of the reagent, and its mode of employment, and also the position of the alkyl groups in the

molecule, have been considered. The subject is still under investigation, but the facts which have so far emerged are recorded in the table, which makes it apparent that an

Structure of reactant	Product obtained by using : Selenium (330—350°)	Pd–C (260—280°) and S (230—240°)
XIII; R = Et; R′ = R″ = R‴ = Me	XVI; R = H; R′ = R″ = R‴ = Me	XVI; R = Et; R′ = R″ = R‴ = Me
XIV; " "	XVI; " "	XVI; " "
XIII; R = R′ = R″ = R‴ = Me	XVI; R = R′ = R″ = R‴ = Me	—
XIV; " "	—	XVI; R = R′ = R″ = R‴ = Me *
XVI; R = Et; R′ = R″ = R‴ = Me	XVI; R = H; R′ = R″ = R‴ = Me	—
XIII; R = Et; R′ = R‴ = Me; R″ = H	XVI; R = R″ = H; R′ = R‴ = Me	XVI; R = Et; R′ = R‴ = Me; R″ = H ‡
XIV; " "	—	XVI; " " *
XVI; " "	XVI; R = Et; R′ = R‴ = Me; R″ = H	—
XIII; R = Et; R′ = H; R″ = R‴ = Me	XVI; R = Et; R′ = H; R″ = R‴ = Me	XVI; R = Et; R′ = H; R″ = R‴ = Me *
XIII; R = Et; R′ = Me; R″ = H; R‴ = OMe	XVI; R = R″ = H; R′ = Me; R‴ = OMe	XVI; R = Et; R′ = Me; R″ = H; R‴ = OMe *
XIII; R = Et; R′ = Me; R″ = H; R‴ = OH	← Unsatisfactory results →	
XIV; R = Et; R′ = Me; R″ = H; R‴ = OMe	XVI; R = R″ = H; R′ = Me; R‴ = OMe	—
XIII; R = Et; R′ = R″ = R‴ = H	Not dehydrogenated	XVI; R = Et; R′ = R″ = R‴ = H †
XIV; " "	XVI; R = Et; R′ = R″ = R‴ = H	—

* Pd–C only. † Sulphur only. ‡ Also with Pd–C at 330—350°.

ethyl group (R) attached to carbon atom no. 1 is eliminated, during dehydrogenation with selenium, from di- and tetra-hydronaphthalenes in which a methyl group is located at carbon atom no. 8. The two examples mentioned earlier by us (*J.*, 1950, 1781) also fall into this category. It also emerges that the ethyl group (R) is retained when dehydrogenation is performed with palladised charcoal and sulphur, and that a methyl group at carbon atom no. 1 is retained under all conditions investigated.



We have also found that when 5-ethyl-1 : 4-dimethyl- and 8-ethyl-1 : 2 : 4-trimethylnaphthalene are heated with selenium at 340° only the latter loses its ethyl group.

In contrast to the majority of the results recorded in the literature, the yields of product obtained in this investigation from reactions in which an alkyl group is eliminated are generally very good. Although there is some indication (*e.g.*, colour of picrates) that the dehydrogenated product is a mixture, the de-alkylated naphthalene is always the major constituent and all attempts to isolate the higher homologue have been unsuccessful.

It is too early to reach any final decisions on the mechanism of the loss of an ethyl group from the systems mentioned. We are extending the investigation to compounds in which groups other than methyl and ethyl are located at the 1- and the 8-position in hydrogenated naphthalenes, and to other ring systems.

The hydrocarbons of structure (XIII) were prepared by reaction of the appropriate Grignard reagent with the tetralones (XV), followed by dehydration and hydrogenation. The tetralones were prepared by conventional methods:

$$\text{Aromatic hydrocarbon} + \text{succinic anhydride} \xrightarrow{AlCl_3} \text{substituted } \beta\text{-benzoylpropionic acid} \xrightarrow[HCl]{Zn\text{–}Hg} \text{substituted } \gamma\text{-phenylbutyric acid} \xrightarrow[via\ \text{acid chloride or } H_2SO_4]{} \text{tetralone.}$$

Attempts were made to prepare 1 : 2 : 3 : 4-tetrahydro-1-keto-5 : 8-dimethoxynaphthalene (XV; R′ = R‴ = OMe, R″ = H). γ-(2 : 5-Dimethoxyphenyl)butyric acid (Fieser, Gates, and Kilmer, *J. Amer. Chem. Soc.*, 1940, **62**, 2966) gave only a tar, with either concentrated or 80% sulphuric acid, presumably as a result of demethylation and subsequent oxidation. Attempted cyclisation of the acid chloride with aluminium chloride or bromide gave a small amount of the desired product, characterised by its semicarbazone. On use of a solution of boron trifluoride in ether as catalyst, a tetralone was obtained having the composition of a hydroxymethoxytetralone. It gave a semicarbazone and a dark green ferric reaction, the product being soluble in ether; but it could not be methylated. From these properties it seems probable that the tetralone is 5 : 6 : 7 : 8-tetrahydro-8-keto-4-methoxy-1-naphthol (XV; R′ = OH, R″ = H, R‴ = OMe).

Experimental

(Analyses by Drs. Weiler and Strauss.)

Unless otherwise stated, the standard conditions for dehydrogenation were as follows: (1) *Palladised charcoal.* Equal weights of catalyst and hydrocarbon were heated at 260—280° for 4—4½ hours. (2) *Selenium.* The hydrocarbon was heated with three times its weight of reagent at 330—350° for 4—4½ hours. All hydrocarbons were distilled from sodium for analysis.

1 : 2 : 3 : 4-*Tetrahydro*-1-*keto*-5 : 7 : 8-*trimethylnaphthalene* (XV; R′ = R″ = R‴ = Me).—β-(2 : 4 : 5-Trimethylbenzoyl)propionic acid, obtained from ψ-cumene, had m. p. 105—107° (Muhr, *Ber.*, 1895, **28**, 3215, gave m. p. 98°; Claus, *Ber.*, 1887, **20**, 1378, gave m. p. 105°). Its *semicarbazone*, needles (from methyl alcohol), had m. p. 184° (Found: C, 61·0; H, 6·55. $C_{14}H_{19}O_3N_3$ requires C, 60·65; H, 6·85%), and its *methyl* ester, a low-melting solid, had b. p. 197°/15 mm. (Found: C, 71·7; H, 7·8. $C_{14}H_{18}O_3$ requires C, 71·8; H, 7·7%). γ-(2 : 4 : 5-Trimethylphenyl)butyric acid had m. p. 120—121° (Willgerodt and Scholtz, *J. pr. Chem.*, 1910, **81**, 391, give m. p. 71°) (Found: C, 75·35; H, 8·95. Calc. for $C_{13}H_{18}O_2$: C, 75·7; H, 8·75%). The butyric acid (12 g.), heated for 1·3 hours on the water-bath with a mixture of sulphuric acid (38 c.c.) and water (14 c.c.), gave the required *tetralone* (10·9 g.; m. p. 58—62°), which crystallised from methyl alcohol as rhombic plates, m. p. 61—62° (Found: C, 82·9; H, 8·05. $C_{13}H_{16}O$ requires C, 83·0; H, 8·5%) (semicarbazone, m. p. 200—201°). A *dibromo*-compound, made in ether with excess of bromine, crystallised from methyl alcohol as needles, m. p. 102—103° (Found: C, 44·9; H, 4·2. $C_{13}H_{14}OBr_2$ requires C, 45·1; H, 4·1%).

1 : 2 : 3 : 4-*Tetrahydro*-5 : 7 : 8-*trimethylnaphthalene*, obtained by reduction of the tetralone (0·5 g.) with amalgamated zinc and hydrochloric acid, crystallised in leaflets (0·25 g.), m. p. 46—47°, from ether–methyl alcohol (Found: C, 89·2; H, 10·6. $C_{13}H_{18}$ requires C, 89·65; H, 10·35%). This was heated with palladised charcoal, to give the known 1 : 2 : 4-trimethylnaphthalene, m. p. 50—52° (Ruzicka and Ehmann, *Helv. Chim. Acta*, 1932, **15**, 140).

8-*Ethyl*-5 : 6-*dihydro*-1 : 2 : 4-*trimethylnaphthalene*.—The above tetralone (7·5 g.) in ether (75 c.c.) was added to ethylmagnesium iodide, from ethyl iodide (33·9 c.c.) and magnesium (21·4 g.) in ether (125 c.c.), and the mixture refluxed for 1 hour and set aside overnight. After decomposition, the *dihydro*-compound (5·8 g.) distilled as a colourless oil, b. p. 145—146°/10 mm. (Found: C, 89·9; H, 10·1. $C_{15}H_{20}$ requires C, 90·0; H, 10·0%).

1-*Ethyl*-1 : 2 : 3 : 4-*tetrahydro*-5 : 7 : 8-*trimethylnaphthalene*.—The previous compound (3·5 g.) in ethyl acetate (75 c.c.) was quantitatively reduced over Adams's platinic oxide catalyst (420 mg.). The *tetrahydro*-compound obtained (3·2 g.; m. p. 48—50°) recrystallised from methyl alcohol–ether in rhombic plates, m. p. 58·5—59·5° (Found: C, 88·8; H, 10·8. $C_{15}H_{22}$ requires C, 89·1; H, 10·9%).

8-*Ethyl*-1 : 2 : 4-*trimethylnaphthalene* (XVI; R = Et, R′ = R″ = R‴ = Me).—The previous compound (0·3 g.) was dehydrogenated with palladised charcoal, and the product was extracted with methyl alcohol and distilled twice. The *hydrocarbon* was converted into its picrate,

from which it was regenerated as an oil (0·17 g.), b. p. 144—145°/8 mm. (Found: C, 90·8; H, 8·9. $C_{15}H_{18}$ requires C, 90·9; H, 9·1%). Its *picrate*, dark red needles, m. p. 97—99° (Found: C, 58·4; H, 5·2. $C_{21}H_{21}O_7N_3$ requires C, 59·0; H, 4·9%), *trinitrobenzene* adduct, long, slender, orange needles, m. p. 124—125° (Found: C, 61·1; H, 5·2. $C_{21}H_{21}O_6N_3$ requires C, 61·3; H, 5·1%), and *trinitrotoluene* adduct, orange needles, m. p. 42—43° (Found: C, 62·1; H, 5·35. $C_{22}H_{23}O_6N_3$ requires C, 62·1; H, 5·4%), were all recrystallised from methyl alcohol.

This naphthalene (0·77 g.) was also obtained when its dihydro-compound (1·0 g.) was dehydrogenated with palladised charcoal.

Dehydrogenation of the tetrahydro-compound (1·6 g.) and of the dihydro-compound (1·5 g.) with the calculated quantities of sulphur, during 3 hours at 220—240°, also yielded (XVI; R = Et, R′ = R″ = R‴ = Me) (1·3 g. and 1·1 g., respectively).

Dehydrogenation of the Tetrahydro-compound with Selenium.—The pure tetralin (0·55 g.) yielded a product, strongly smelling of alkyl selenide. It was extracted with ether and distilled (0·39 g.), giving an oil, b. p. 129—135°/5 mm. Its picrate, after two crystallisations from methyl alcohol, formed orange needles, m. p. 147—148°, undepressed by the picrate of authentic 1 : 2 : 4-trimethylnaphthalene (see above; Ruzicka and Ehmann, *loc. cit.*). It was also characterised through its trinitrobenzene adduct, m. p. 165° (*idem, ibid.*). Its *trinitrotoluene* adduct crystallised as lemon-yellow needles (from methyl alcohol), m. p. 94° (Found: C, 60·2; H, 4·7. $C_{20}H_{19}O_6N_3$ requires C, 60·5; H, 4·8%).

Dehydrogenation of the dihydro-compound (2·9 g.) with selenium (8·7 g.) under the above conditions similarly yielded 1 : 2 : 4-trimethylnaphthalene (1·54 g.).

Action of Selenium on 8-Ethyl-1 : 2 : 4-trimethylnaphthalene.—The naphthalene (0·6 g.), heated with selenium (1·8 g.) under the above conditions, gave 1 : 2 : 4-trimethylnaphthalene (0·23 g.) and a strong smell of alkyl selenide. The product gave a picrate, m. p. 126—132°, raised to 147° by three crystallisations from methyl alcohol.

5 : 6-*Dihydro*-1 : 2 : 4 : 8-*tetramethylnaphthalene*, prepared from the tetralone (XV; R′ = R″ = R‴ = Me) (6 g.) and methylmagnesium iodide (from methyl iodide, 20 c.c.), was obtained as a colourless oil (2·9 g.), b. p. 140—148°/9—12 mm. (Found: C, 90·4; H, 9·5. $C_{14}H_{18}$ requires C, 90·35; H, 9·65%). The corresponding tetrahydro-compound was obtained by quantitative reduction of the preceding compound over palladised charcoal in ethyl acetate. The tetrahydro-compound was not analysed.

Dehydrogenation. The tetrahydro-compound (0·7 g.) was heated with selenium, yielding 1 : 2 : 4 : 8-tetramethylnaphthalene (0·55 g.), m. p. 56—57° (Found: C, 91·3; H, 8·8. Calc. for $C_{14}H_{16}$: C, 91·3; H, 8·7%) (Ruzicka, Ehmann, and Mörgeli, *Helv. Chim. Acta*, 1933, **16**, 314, describe this compound as an oil), depressed by authentic 1 : 2 : 4-trimethylnaphthalene, m. p. 54—55°. Its picrate had m. p. 149—149·5° (Ruzicka, Ehmann, and Mörgeli, *loc. cit.*, give m. p. 145·5°) (Found: C, 58·0; H, 4·9. Calc. for $C_{20}H_{19}O_7N_3$: C, 58·1; H, 4·6%). Its *trinitrobenzene* adduct crystallised from methyl alcohol as deep yellow rods, m. p. 167° (Found: C, 60·2; H, 4·6. $C_{20}H_{19}O_6N_3$ requires C, 60·4; H, 4·8%). Its *trinitrotoluene* adduct formed yellow needles, m. p. 88° (Found: C, 61·1; H, 4·75. $C_{21}H_{21}O_6N_3$ requires C, 61·3; H, 5·1%).

Dehydrogenation of the corresponding dihydro-compound (0·5 g.) with palladised charcoal under conditions mentioned above again gave 1 : 2 : 4 : 8-tetramethylnaphthalene (0·26 g.).

(With Dr. D. R. A. Whyte). 1 : 2 : 3 : 4-*Tetrahydro*-1-*keto*-5-*methoxy*-8-*methylnaphthalene* (cf. Cocker, Lipman, and Whyte, *Chem. and Ind.*, 1950, 237).—β-(2-Methoxy-5-methylbenzoyl)-propionic acid, m. p. 114—115·5°, obtained from methyl *p*-tolyl ether and succinic anhydride in nitrobenzene, was reduced under Clemmensen conditions to γ-(2-methoxy-5-methylphenyl)butyric acid, b. p. 180—185°/4 mm., m. p. 63·5—64·5°. The butyric acid (11 g.) and pure thionyl chloride (60 c.c.) were warmed until molten, set aside for 30 minutes, and then refluxed for 10 minutes. The excess of thionyl chloride was removed at the pump. The residue was cooled in ice, dissolved in carbon disulphide (50 c.c.), and treated with powdered aluminium chloride (10 g.) in one portion. After refluxing for 10 minutes, the mixture was cooled and poured on ice and hydrochloric acid. From the organic layer, the required *tetralone* * was obtained as an oil (7·9 g.), b. p. 157—158°/9 mm., which solidified on storage and had m. p. 31—33° (Found: C, 76·1; H, 7·7. $C_{12}H_{14}O_2$ requires C, 75·8; H, 7·4%); the *semicarbazone* * (rosettes of needles from aqueous alcohol) melted at 233—234° (Found: C, 63·2; H, 6·6. $C_{13}H_{17}O_2N_3$ requires C, 63·2; H, 6·9%).

1 : 2 : 3 : 4-*Tetrahydro*-5-*methoxy*-8-*methylnaphthalene.*—The above tetralone (2·5 g.) was reduced with amalgamated zinc (50 g.), hydrochloric acid (75 c.c.), and water (32 c.c.) for

* These compounds were described by Cocker, Lipman, and Whyte (*loc. cit.*, where refs. are given; see also Herran *et al.*, *J. Org. Chem.*, 1951, **16**, 899) without preparative details and analyses.

22 hours. The *tetrahydro*-compound (1·14 g.) had b. p. 60—114°/8 mm. and rapidly solidified. It crystallised from aqueous alcohol as white needles or plates, m. p. 43—43·5° (Found: C, 81·4; H, 8·9. $C_{13}H_{16}O$ requires C, 81·8; H, 9·1%).

5-Ethyl-5 : 6 : 7 : 8-tetrahydro-4-methyl-1-naphthol.—1 : 2 : 3 : 4-Tetrahydro-1-keto-5-methoxy-8-methylnaphthalene (7·9 g.), on reaction with ethylmagnesium iodide (from ethyl iodide, 35 c.c.), yielded *1-ethyl-3 : 4-dihydro-5-methoxy-8-methylnaphthalene* as an oil (4·2 g.), b. p. 142—150°/9—10 mm. (Found: C, 81·7; H, 8·9. $C_{14}H_{18}O$ requires C, 83·2; H, 8·9%). When this compound (7·3 g.) was hydrogenated over Raney nickel, and the product heated for 3 hours with hydriodic acid (29·5 c.c.) and acetic acid (29·5 c.c.), it afforded the *tetrahydronaphthol*; this distilled as an oil (3·3 g.; b. p. 160—170°/16 mm.), which solidified and crystallised from light petroleum as needles, m. p. 58—59° (Found: C, 82·4; H, 9·8. $C_{13}H_{18}O$ requires C, 82·1; H, 9·5%); the *carbanilate* (needles from methyl alcohol) had m. p. 156—157° (Found: C, 77·6; H, 7·9. $C_{20}H_{23}O_2N$ requires C, 77·7; H, 7·4%).

Dehydrogenation of the tetrahydronaphthol gave unsatisfactory results, but its methyl ether was smoothly dehydrogenated both with palladised charcoal and with selenium, as follows.

(1) *With palladised charcoal.* The ether (1·5 g.) gave a product which after demethylation yielded 5-ethyl-4-methyl-1-naphthol (0·7 g.), b. p. 126—148°/9 mm. It was not further purified. Its *picrate* crystallised from methyl alcohol as orange-red needles, m. p. 96—97° (Found: C, 55·3; H, 4·0. $C_{19}H_{17}O_8N_3$ requires C, 54·9; H, 4·1%). Its *trinitrobenzene* adduct, golden needles, m. p. 103—104° (Found: C, 56·55; H, 4·3. $C_{19}H_{17}O_7N_3$ requires C, 57·15; H, 4·3%), *trinitrotoluene* adduct, yellow needles, m. p. 54—55° (Found: C, 55·9; H, 4·6. $C_{13}H_{14}O + 1{\cdot}25C_7H_5O_6N_3$ requires C, 55·55; H, 4·3%), and *styphnate*, deep yellow needles, m. p. 131—132° (Found: C, 53·0; H, 3·7. $C_{19}H_{17}O_9N_3$ requires C, 52·9; H, 3·9%), were all crystallised from methyl alcohol.

(2) *With selenium.* The ether (1·5 g.) was dehydrogenated and demethylated, yielding an oily product (0·6 g.) consisting largely of 4-methyl-1-naphthol. Its *picrate* crystallised from methyl alcohol as brick-red needles, m. p. 174—175° (Found: C, 53·0; H, 3·7. $C_{17}H_{13}O_8N_3$ requires C, 52·7; H, 3·3%). Its *trinitrobenzene* adduct crystallised from benzene as orange needles, m. p. 173—174° (decomp.) (Found: C, 54·8; H, 3·4. $C_{17}H_{13}O_7N_3$ requires C, 55·0; H, 3·5%); its *trinitrotoluene* adduct crystallised from benzene–light petroleum as golden needles, m. p. 121—122° (Found: C, 55·8; H, 4·0. $C_{18}H_{15}O_7N_3$ requires C, 56·1; H, 3·9%); its *styphnate* (red needles from methyl alcohol) had m. p. 169° (Found: C, 50·4; H, 3·4. $C_{17}H_{13}O_9N_3$ requires C, 50·6; H, 3·2%). Dehydrogenation of 1-ethyl-3 : 4-dihydro-5-methoxy-8-methylnaphthalene (2·4 g.) with selenium, followed by demethylation, also yielded 4-methyl-1-naphthol (0·6 g.).

1-Ethyl-1 : 2 : 3 : 4-tetrahydro-5 : 8-dimethylnaphthalene.—1 : 2 : 3 : 4-Tetrahydro-1-keto-5 : 8-dimethylnaphthalene was prepared by the method of Barnett and Sanders (*J.*, 1933, 434) or, preferably, by cyclisation of the acid chloride of γ-(2 : 5-dimethylphenyl)butyric acid, prepared from the acid (26 g.) and thionyl chloride (26 c.c.) which were heated until molten, set aside for 30 minutes, and then warmed for 10 minutes on the water-bath. The acid chloride (19 g.) distilled as a colourless liquid, b. p. 134—142°/2—5 mm. It was dissolved in carbon disulphide (215 c.c.) and treated with aluminium chloride (22·8 g.), added in one portion, with cooling, giving the required tetralone (11·3 g.), b. p. 138—142°/4 mm., m. p. 30—33° (cf. Barnett and Sanders, *loc. cit.*; Ruzicka and Waldman, *Helv. Chim. Acta*, 1932, **15**, 907, 914; Baddeley, *J.*, 1944, 232). From this tetralone (4·7 g.), *1-ethyl-3 : 4-dihydro-5 : 8-dimethylnaphthalene* was obtained in the usual manner as a colourless oil (3·8 g.), b. p. 140°/23 mm. (Found: C, 90·3; H, 9·6. $C_{14}H_{18}$ requires C, 90·3; H, 9·7%). When this dihydro-compound (1·7 g.) was reduced in glacial acetic acid over palladised charcoal, it gave *1-ethyl-1 : 2 : 3 : 4-tetrahydro-5 : 8-dimethylnaphthalene* as a colourless oil (1·1 g.), b. p. 150°/5 mm., m. p. 35—36·5° (Found: C, 89·1; H, 10·8. $C_{14}H_{20}$ requires C, 89·3; H, 10·6%).

Dehydrogenation. (1) With palladised charcoal. The tetralin (0·4 g.), treated under the standard conditions, yielded *5-ethyl-1 : 4-dimethylnaphthalene* (XVI; R = Et, R′ = R‴ = Me, R″ = H) as an oil which, on regeneration from its picrate, distilled (0·1 g.), b. p. 145—149°/6 mm. (Found: C, 91·3; H, 8·8. $C_{14}H_{16}$ requires C, 91·3; H, 8·7%). Its *picrate* crystallised from methyl alcohol as orange-red needles, m. p. 83° (Found: C, 58·4; H, 4·6. $C_{20}H_{19}O_7N_3$ requires C, 58·1; H, 4·6%), or from benzene–light petroleum with m. p. 78° (Found: C, 61·7; H, 5·0. $C_{20}H_{19}O_7N_3,0{\cdot}5C_6H_6$ requires C, 61·1; H, 4·0%). Its *trinitrobenzene* adduct consisted of golden needles (from methyl alcohol), m. p. 115° (Found: C, 59·8; H, 4·7. $C_{20}H_{19}O_6N_3$ requires C, 60·4; H, 4·8%).

Palladised charcoal at 330—350° and sulphur at 230—250° gave a similar result. 5-Ethyl-

1 : 4-dimethylnaphthalene was also obtained from 1-ethyl-3 : 4-dihydro-5 : 8-dimethylnaphthalene and palladised charcoal under the standard conditions.

(2) With selenium. The tetrahydro-compound (0·7 g.) yielded 1 : 4-dimethylnaphthalene (0·5 g.), b. p. 120—125°/15 mm., which gave a picrate, m. p. 141—142°, and trinitrobenzene adduct, m. p. 164° (Elsevier's Encyclopædia, 1948, **12**, *B*, 136, gives m. p. 143—144° and 165—166°, respectively).

Non-action of Selenium on 5-Ethyl-1 : 4-dimethylnaphthalene.—The naphthalene was recovered unchanged after being heated with selenium at 330—340°, and very little smell of alkyl selenide was evolved.

1-Ethyl-3 : 4 dihydro-5 : 7-dimethylnaphthalene was obtained (3·9 g.) as an oil, b. p. 128—134°/8—10 mm. (Found : C, 89·95; H, 9·95. $C_{14}H_{18}$ requires C, 90·3; H, 9·7%), when 1 : 2 : 3 : 4-tetrahydro-1-keto-5 : 7-dimethylnaphthalene (Barnett and Sanders, *loc. cit.*; Forrest, Tucker, and Whalley, *J.*, 1949, 3194) (5·7 g.) was treated with ethylmagnesium iodide (from ethyl iodide, 15·8 c.c.). Reduction (3·1 g.) in methyl alcohol over Raney nickel gave *1-ethyl-1 : 2 : 3 : 4-tetrahydro-5 : 7-dimethylnaphthalene* (2·35 g.), b. p. 124—129°/9 mm. (Found : C, 89·4; H, 10·4. $C_{14}H_{20}$ requires C, 89·35; H, 10·65%).

5-Ethyl-1 : 3-dimethylnaphthalene (XVI; R = Et, R′ = H, R″ = R‴ = Me).—This *hydrocarbon* was obtained (1·0 g.) when the previous compound (1·2 g.) was dehydrogenated under standard conditions with either palladised charcoal or selenium. Regenerated from its picrate, it distilled as an oil, b. p. 131°/9 mm. (Found : C, 91·3; H, 8·7. $C_{14}H_{16}$ requires C, 91·3; H, 8·7%). Its *picrate*, orange-red needles, m. p. 109—110° (Found : C, 58·3; H, 4·9. $C_{20}H_{19}O_7N_3$ requires C, 58·1; H, 4·6%), *trinitrobenzene* adduct, yellow needles, m. p. 118° (Found : C, 60·6; H, 4·7. $C_{20}H_{19}O_6N_3$ requires C, 60·45; H, 4·8%), *styphnate*, orange needles, m. p. 115—116° (Found : C, 55·6; H, 4·5. $C_{20}H_{19}O_8N_3$ requires C, 55·95; H, 4·4%), and *trinitrotoluene* adduct, yellow needles, m. p. 70—71° (Found : C, 61·35; H, 5·0. $C_{21}H_{21}O_6N_3$ requires C, 61·3; H, 5·1%), were all recrystallised from methyl alcohol.

1-Ethyl-3 : 4-dihydronaphthalene, obtained from 1-tetralone, was an oil, b. p. 96—98°/6 mm. (Found : C, 91·3; H, 8·8. $C_{12}H_{14}$ requires C, 91·1; H, 8·9%).

5 : 6 : 7 : 8-Tetrahydro-8-keto-4-methoxy-1-naphthol (XV; R′ = OH, R″ = H, R‴ = OMe).—γ-(2 : 5-Dimethoxyphenyl)butyric acid (Fieser, Gates, and Kilmer, *loc. cit.*) (2·8 g.) in benzene (15 c.c.) was treated with boron trifluoride (48% in ether, 12 c.c.) and set aside overnight. After decomposition in the usual way, the ethereal extract was shaken with methyl sulphate and sodium hydroxide, dried, and distilled, giving the *tetrahydronaphthol* as a thick oil (0·5 g.), b. p. 156—160°/5 mm. It solidified, and crystallised from light petroleum as long, yellow needles, m. p. 95° (Found : C, 68·8; H, 6·5. $C_{11}H_{12}O_3$ requires C, 68·8; H, 6·25%); the *semicarbazone* had m. p. 252—254° (decomp.) (Found : C, 58·2; H, 5·95. $C_{12}H_{15}O_3N_3$ requires C, 57·85; H, 6·0%).

1 : 2 : 3 : 4-Tetrahydro-1-keto-5 : 8-dimethoxynaphthalene (XV; R′ = R‴ = OMe, R″ = H).—Cyclisation of the butyric acid (2·8 g.), through its chloride (by use of 2·9 g. of phosphorus pentachloride in 12 c.c. of benzene) followed by stannic chloride (3 c.c.), yielded a dark red oil (0·5 g.), b. p. 200—240°/5 mm. It formed a *semicarbazone*, m. p. 185·5—186·5° (Found : C, 59·3; H, 6·2. $C_{13}H_{17}O_3N_3$ requires C, 59·3; H, 6·5%).

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18. *Triterpenoids. Part V.* Some Relative Configurations in Rings* C, D, *and* E *of the* β*-Amyrin and the Lupeol Group of Triterpenoids.*

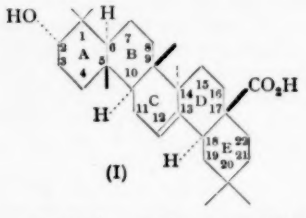
By D. H. R. Barton and N. J. Holness.

Relative configurations have been assigned to asymmetric centres at positions 10, 11, 12, 13, 14, 16, 17, 18, 19, and 20 in the β-amyrin group of triterpenoids. 18-*iso*Oleanolic acid, methyl olean-10-enolate acetate, methyl 11-keto-oleananolate acetate and a number of other derivatives of oleanolic acid have been prepared. The lactonisation of oleanolic and morolic acids has been investigated.

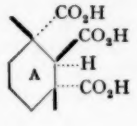
The synthesis of "betulin triol diacetate," starting with siaresinolic acid, is reported. This has enabled configurations to be assigned at $C_{(13)}$ and $C_{(18)}$ in the lupeol group of triterpenoids. Together with earlier work, this permits relative configurations to be assigned also at $C_{(10)}$, $C_{(14)}$, and $C_{(19)}$.

Some experiments in the ursolic acid series are reported.

When this work on the stereochemistry of the pentacyclic triterpenoids was initiated knowledge of the subject was meagre. Giacomello (*Gazzetta*, 1938, **68**, 363) had reported an *X*-ray analysis of certain members of the β-amyrin group from which he had concluded that all five rings were fused *trans-anti-trans* to each other. This would imply the stereochemical relationships summarised in the formula (I) for oleanolic acid. The almost ubiquitous hydroxyl group at $C_{(2)}$ was placed by Giacomello as indicated in (I) and this assignment of configuration was tentatively confirmed by Ruzicka and Gubser (*Helv. Chim. Acta*, 1945, **28**, 1054) from a study (*a*) of the rates of alkaline hydrolysis of β-amyrin acetate and *epi*-β-amyrin acetate and (*b*) of molecular models of the Stuart type. However, as shown in the sequel, Giacomello's conclusions are incorrect in at least two respects.



(I)



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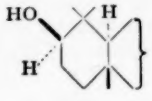

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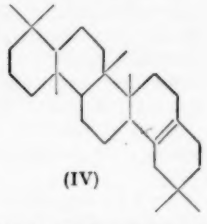


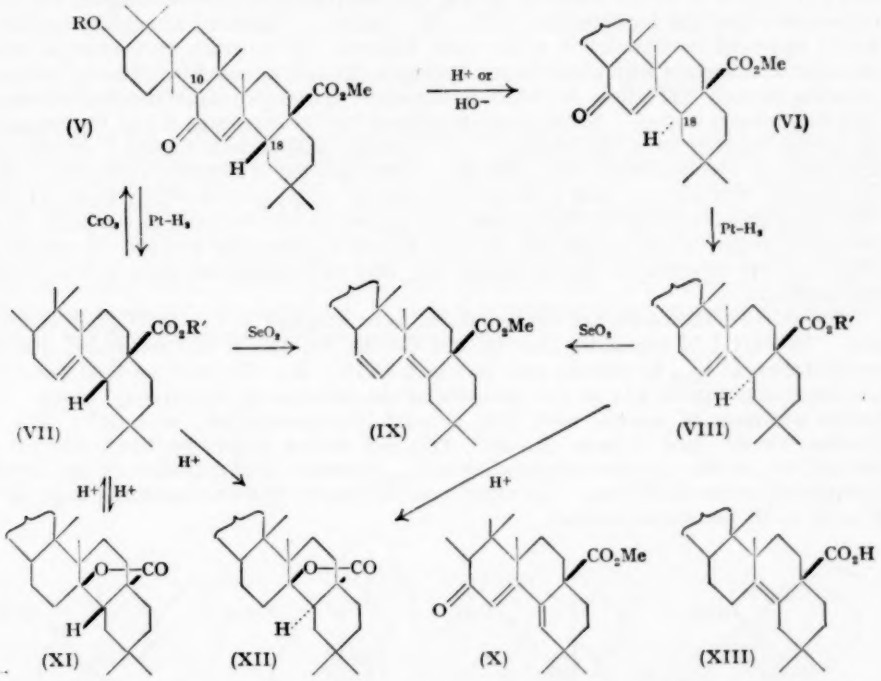
(IV)

A study of the properties of the $C_{11}H_{16}O_6$ tricarboxylic acid obtained by vigorous oxidation of abietic acid enabled Barton and Schmeidler (*J.*, 1948, 1197; 1949, S 232) to conclude that the C_{11} acid had the configuration shown in (II). In view of the relations which have been established between abietic acid and other diterpenoids on the one hand and between abietic acid and the triterpenoids on the other (for summaries see Barton, *Quart. Reviews*, 1949, **3**, 36; Jeger, "Fortschritte der Chemie organischer Naturstoffe," 1950, Vol. VII) it can be concluded that, provided that there is no inversion during the genesis of (II), rings A and B of oleanolic acid are fused *trans* to each other. With regard to the configuration of the hydroxyl at $C_{(2)}$ consideration of (*a*) the relative stabilities of the β- and *epi*-β-amyrin, (*b*) the relative degree of steric hindrance in the two alcohols, and (*c*) elimination evidence, all considered in the light of the concept of equatorial and polar bonds (see Barton and Rosenfelder *J.*, 1951, 1048), enabled one of us (Barton, *Experientia*, 1950, **6**, 316) to conclude that this hydroxyl group had the *opposite* configuration in β-amyrin and its congeners to that assigned by Giacomello and by Ruzicka. The stereochemical data for ring A in the β-amyrin series relative to an arbitrary configuration at $C_{(5)}$ can, then, be summarised as in partial formula (III).

As bearing on the practical work now reported it is convenient to turn next to the stereochemistry of rings D and E in oleanolic acid. As has been emphasized previously

* Part IV, *J.*, 1951, 3147.

(Barton and Brooks, *J.*, 1951, 257; Davy, Halsall, and Jones, *Chem. and Ind.*, 1951, 233) the carboxyl group in this acid must be polar in the stereochemical sense. This conclusion, however, still permits *cis*- or *trans*-fusion of rings D and E. The argument of Bilham and Kon (*J.*, 1940, 1469) that this fusion is *cis* would appear to be invalidated by the new formulation (IV) (Barton and Brooks, *loc. cit.*) for oleanene II. We have provided a final solution to the problem by the reactions summarised below (see also Barton and Holness, *Chem. and Ind.*, 1951, 233). Kitasato (*Acta Phytochim.*, 1934, **8**, 1) found that methyl 11-keto-oleanolate acetate (V; R = Ac) was isomerised to methyl ψ-11-keto-oleanolate acetate (VI; R = Ac) by treatment with hydrogen bromide in acetic acid. We find that this change can also be effected by alkali (cf. the corresponding isomerisation in the β-amyrin series; Picard and Spring, *J.*, 1940, 1198; Ruzicka, Müller, and Schellenberg, *Helv. Chim. Acta*, 1939, **22**, 758; Budziarek, Johnston, Manson, and Spring, *Chem. and Ind.*, 1951, 478). Clearly the asymmetric centres at $C_{(10)}$ and/or $C_{(18)}$ are inverted by enolisation. Just as catalytic hydrogenation of methyl 11-keto-oleanolate acetate gives methyl oleanolate acetate (VII; R = Ac, R′ = Me), so the similar reduction of methyl ψ-11-keto-oleanolate acetate gave the methyl ester acetate, m. p. 250—252°, $[\alpha]_D$ + 44°, of a new triterpenoid hydroxy-acid. This was shown to be methyl 18-*iso*oleanolate acetate

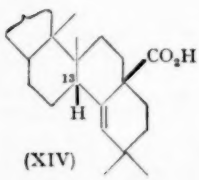


(VIII; R = Ac, R′ = Me), differing from ordinary methyl oleanolate acetate only in its configuration at $C_{(18)}$, for on oxidation by selenium dioxide it gave methyl dehydro-oleanolate acetate (IX; R = Ac) (see Barton and Brooks, *loc. cit.*), prepared in a similar way (Ruzicka, Grob, and van der Sluys-Veer, *Helv. Chim. Acta*, 1939, **22**, 788) by oxidation of methyl oleanolate acetate. This shows that the configuration at $C_{(18)}$ in oleanolic acid is unstable; therefore, the D–E fusion must be *cis* (see further discussion below). The formation of (IX; R = Ac) by reduction with sodium and alcohol and subsequent acetylation of both (V; R = Ac) and (VI; R = Ac) is further proof (Kitasato, *loc. cit.*) for the postulated inversion at $C_{(18)}$ but *not* at $C_{(10)}$. Further evidence supporting this conclusion is the fact that the dienone (X; R = Ac), where $C_{(18)}$ is no longer asymmetric, cannot be

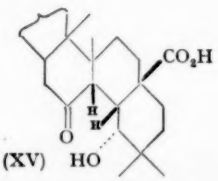
isomerised by alkali (see Experimental). These experiments also imply that rings B and C must be fused in the more stable arrangement. An even more convincing proof of this has been given recently by Budziarek *et al.* (*loc. cit.*).

Additional, and more subtle, evidence for our conclusion that rings D and E are *cis*-fused in oleanolic acid was obtained in the following way. Oleanolic acid (VII; R = R′ = H), on treatment in chloroform solution with a stream of hydrogen chloride, gave an equilibrium mixture of the free acid (76%) and its true lactone (XI; R = H) (24%), the same equilibrium mixture being obtained on treating the lactone itself in the same way. The equilibrium appears to be set up almost instantaneously and therefore the reaction velocity both to *and from* (XI; R = H) must be large. Now it has been shown conclusively (Davy, Halsall, and Jones, *J.*, 1951, 458) that, thermodynamically, the double bond position in δ-oleanolic acid (XIII; R = H) must be much more stable than in oleanolic acid itself (VII; R = R′ = H) or in 18-*iso*oleanolic acid (VIII; R = R′ =H). Since the composition of the equilibrium mixture of oleanolic acid and its lactone remains unchanged even after 17 hour's treatment with chloroformic hydrogen chloride it is clear that the rate of opening of the lactone ring to give the more stable (XIII; R = H) must be very much slower (by a factor of at least 10^4) than the opening to regenerate oleanolic acid (VII; R = R′ = H). In our opinion this remarkable difference demands the steric explanation that the formation of (XIII; R = H) is not favoured kinetically because it would represent *cis*-elimination in an *ionic* reaction. In contrast, treatment of 18-*iso*-oleanolic acid acetate with chloroformic hydrogen chloride afforded irreversibly the corresponding lactone (XII; R = Ac) which is, therefore, much more stable thermodynamically than the ordinary lactone. Surprisingly, this 18-*iso*-lactone acetate is, in fact, the compound frequently referred to in the literature as " oleanolic acid lactone acetate " (Winterstein and Stein, *Z. physiol. Chem.*, 1931, **199**, 64). Thus reaction of oleanolic acid acetate, or of the derived 18-*n*-lactone, with hydrogen bromide in acetic acid or with refluxing hydrochloric–acetic acid—reagents which have a far greater proton-donating power than chloroformic hydrogen chloride—afforded the long known 18-*iso*lactone acetate. A number of other lactones reported in the literature, *e.g.*, that of hederagenin, must also be 18-*iso*-compounds.

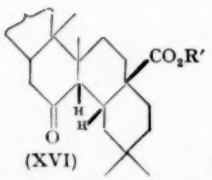
The above evidence enables configurations to be assigned to $C_{(17)}$ and $C_{(18)}$ in oleanolic acid. In Part I of this series (Barton and Brooks, *loc. cit.*), it was concluded that the configuration at $C_{(13)}$ in morolic acid was such (XIV; R = H) that the C–H bond was stereochemically polar and on the *same* side of the molecule as the carboxyl group. The partial synthesis of morolic acid from dihydro-12-ketosiaresinolic acid (XV; R = H) (Barton, Brooks, and Holness, *J.*, 1951, 277) (see further below) by Wolff–Kishner reduction, etc., shows that the configuration at $C_{(13)}$ in morolic acid must also be the thermodynamically more stable one. The same must be true in 12-keto-oleananolic acid (XVI; R = R′ = H) (see Experimental).



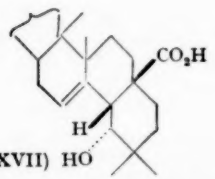
(XIV)



(XV)



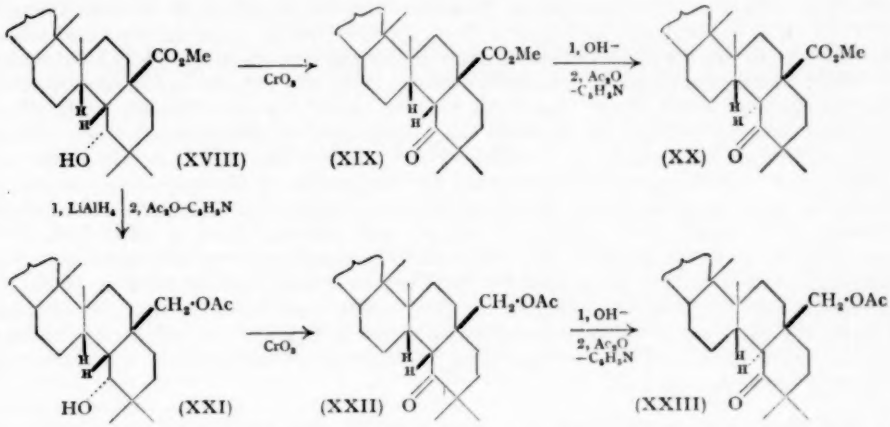
(XVI)



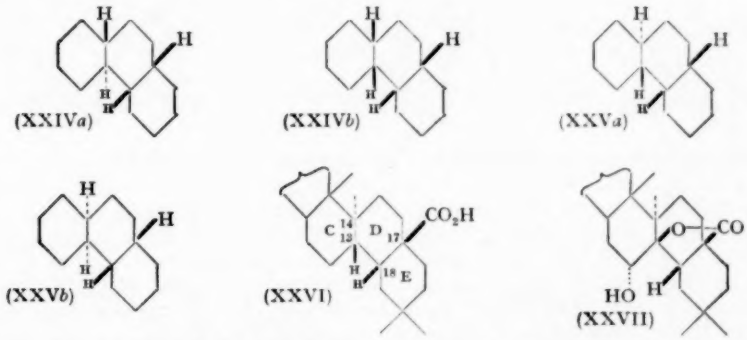
(XVII)

Our attention was directed next to the stereochemistry of rings D and E in siaresinolic acid (XVII; R = H). This acid has been related to oleanolic acid (Bilham, Kon, and Ross, *J.*, 1942, 540; Ruzicka, Grob, Egli, and Jeger, *Helv. Chim. Acta*, 1943, **26**, 1218), but only in ways which destroy the configuration at $C_{(18)}$. Siaresinolic acid was converted into methyl dihydrosiaresinolate monoacetate (XVIII; R = Ac) as described previously (Barton, Brooks, and Holness, *loc. cit*). Oxidation by chromic acid gave the corresponding ketone (XIX; R = Ac). This was shown to be a *cis*-α-decalone, for on treatment with methanolic potassium hydroxide followed by reacetylation it afforded an isomeric *trans*-

α-decalone (XX; R = Ac). In confirmation, reduction of (XVIII; R = Ac) by lithium aluminium hydride followed by reacetylation furnished the diacetate (XXI; R = Ac). This was oxidised by chromic acid to a ketone (XXII; R = Ac), which was isomerised by methanolic potassium hydroxide and then reacetylated to give the *trans*-α-decalone



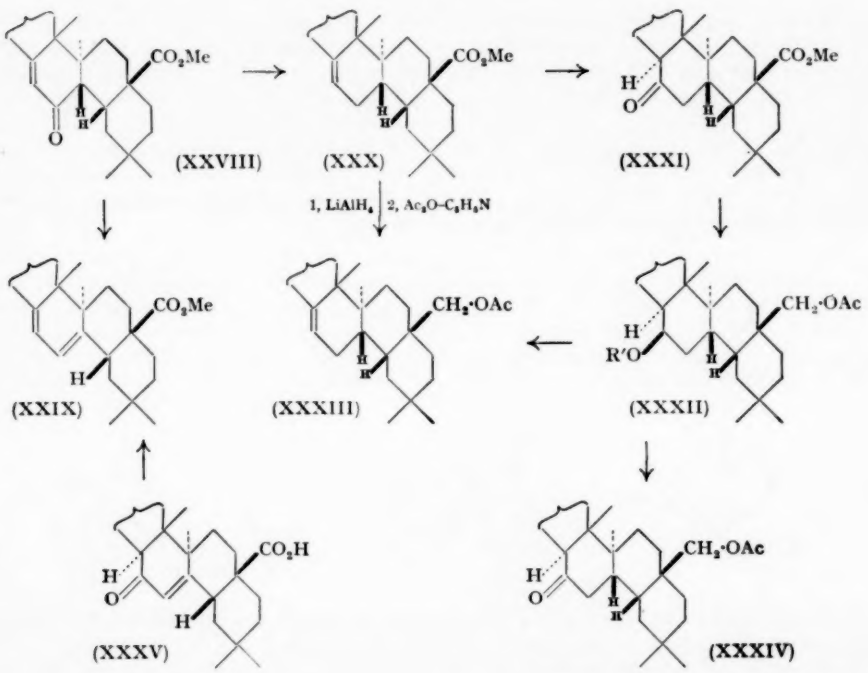
(XXIII; R = Ac). Now the properties of the hydroxyl group at $C_{(19)}$ in siaresinolic acid, in particular its resistance to esterification and its ease of elimination, show that it must be polar in the stereochemical sense (cf. Barton, *Experientia*, *loc. cit.*). The ease of elimination shows further (Barton, *loc. cit.*) that the $C_{(18)}$ hydrogen atom must also be polar and *trans* to the $C_{(19)}$-hydroxyl group. All these facts are accommodated by (XVII), (XVIII), etc. Incidentally, it can be argued from the failure of the carboxyl group of siaresinolic acid to lactonise with the $C_{(19)}$-hydroxyl group that the latter must, if polar, be on the side of ring E opposite to the carboxyl group. This is only possible if rings D and E are *cis*-fused. Thus all the stereochemical evidence is in agreement.



The experiments described above also appear to provide information on the relative configuration of $C_{(14)}$ with respect to $C_{(17)}$. In compounds (XV) and (XVI) the more stable arrangement of the C–D ring junction is that with the $C_{(13)}$:$C_{(18)}$ relation *syn* and the D–E ring fusion *cis*. By analogy with the important experiments by Linstead and his collaborators (*J.*, 1950, 1428 and earlier papers) as further discussed by W. S. Johnson (*Experientia*, 1951, **7**, 315) in the light of the theory of equatorial and polar bonds, it can be concluded that of the pairs of hydrocarbons, (XXIV*a*) and (XXIV*b*), and (XXV*a*) and (XXV*b*), (XXIV*a*) would be *more* stable than (XXIV*b*), and (XXV*a*) more stable than (XXV*b*). Clearly it is the latter relation which holds for rings C, D, and E in oleananolic

acid. The stereochemistry of rings D and E in this acid can, therefore, be summarised as in (XXVI).

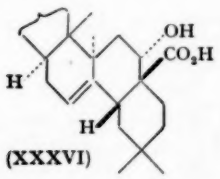
Attention was next directed to derivatives of oleananolic acid substituted at $C_{(12)}$. Ruzicka, Hösli, and Hofmann (*Helv. Chim. Acta*, 1936, **19**, 109) demonstrated that reaction of oleanolic acid acetate with perhydrol in acetic acid afforded a hydroxy-lactone, m. p. 292—294°. Because of its method of formation this compound must be formulated as (XXVII; R = Ac) with the $C_{(13)}$(–O) and the $C_{(17)}$ (–CO–) bonds polar on the same side of the ring system and the $C_{(12)}$(–OH) bond polar and on the opposite side. Picard and Spring (*J.*, 1940, 1387), later prepared a hydroxy-lactone, m. p. 333°, by the action of perbenzoic acid on oleanolic acid acetate. Since this reaction should, on a mechanistic basis, afford the same hydroxy-lactone, we repeated the preparation by both methods and, in fact, obtained the same substance, m. p.s 294—295° and then 325—328° (decomp.) after resolidification. The identity was confirmed by comparison of the respective diacetates. The same hydroxy-lactone was also obtained by potassium permanganate oxidation of oleanolic acid acetate (cf. Aumüller, Wedekind, and Schicke, *Annalen*, 1935, **517**, 211). The 12(polar)-hydroxyl group in this compound is *not* acetylated by cold pyridine–acetic anhydride, but is readily acetylated by this reagent during half an hour at 100°. In agreement with the assigned configuration, compounds which have the opposite, thermodynamically more stable (equatorial) hydroxyl group at $C_{(12)}$ and are prepared by sodium and alcohol reduction of the corresponding 12-ketones, are *more* readily acetylated (cf. Barton, *Experientia, loc. cit.*).



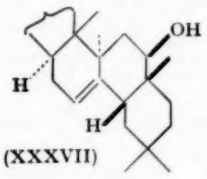
After completion of these experiments concerning $C_{(12)}$ it became important to study similarly reactions at $C_{(11)}$. Methyl 12-keto-oleananolate acetate (XVI; R = Ac, R′ = Me) was converted into the αβ-unsaturated ketone (XXVIII; R = Ac) by dehydrogenation with bromine. Catalytic hydrogenation of this ketone gave a mixture of the homoannular diene (XXIX; R = Ac) and the desired methyl olean-10-enolate acetate (XXX;

R = Ac). Since this mixture was only separable by careful chromatography a more convenient route to (XXX; R = Ac) was sought. This was found in Wolff–Kishner reduction of (XXVIII; R = Ac) followed by methylation and reacetylation. Treatment of methyl olean-10-enolate acetate, which showed an ultra-violet absorption spectrum characteristic of a triply substituted double bond, gave, with perhydrol–acetic acid, methyl 11-keto-oleananolate acetate (XXXI; R = Ac), m. p. 268—270°, $[\alpha]_D$ +3°, $\lambda_{max.}$ 292 mμ, $\varepsilon_{max.}$ = 30 (in alcohol). Lithium aluminium hydride reduction of this ketone, followed by acetylation with pyridine–acetic anhydride at room temperature, furnished the triol diacetate (XXXII; R = Ac, R′ = H). More vigorous acetylation with the same reagent at 100° gave the triacetate (XXXII; R = R′ = Ac). On treatment with phosphorus oxychloride in pyridine the hydroxy-diacetate afforded olean-10-ene-2 : 28-diol diacetate (XXXIII; R = Ac), the constitution of which was confirmed by its preparation by the reduction of (XXX; R = Ac) with lithium aluminium hydride followed by reacetylation. Chromic acid oxidation of 2 : 28-diacetoxyolean-11-ol (XXXII; R = Ac, R′ = H) gave the corresponding ketone (XXXIV; R = Ac). The constitution of (XXIX; R = Ac) was confirmed by its preparation from 11-keto-oleanolic acid acetate (XXXV; R = Ac).

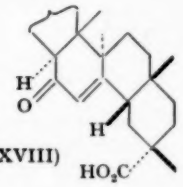
The $C_{(11)}$-hydroxyl group in 2 : 28-diacetoxyolean-11-ol is regarded as polar, (*a*) because of its hindrance to acetylation, (*b*) because of the ease of (*trans*-)elimination of water on treatment with phosphorus oxychloride in pyridine, and (*c*) because of the method of preparation.* Now, as mentioned above, the B–C and C–D ring junctures in oleananolic acid are in the more stable arrangements. Following the arguments of W. S. Johnson (*loc. cit.*), this is to be interpreted as indicating that ring C has a chair conformation. In the chair conformation of *cyclo*hexane two adjacent polar-type substituents must necessarily be *opposite* in configuration (see Barton, *Experientia*, *loc. cit.*). Since the configuration of a $C_{(12)}$-hydroxyl group, if polar, is opposite to the configuration of the $C_{(17)}$-carboxyl group (see above), the configuration of the $C_{(11)}$ polar hydroxyl group must be the *same* as that of the $C_{(17)}$-carboxyl group. Furthermore the $C_{(11)}$ polar hydroxyl group must be *trans* (see above) to the $C_{(10)}$-hydrogen atom and, therefore, the configurations shown in the formulæ (XXXI) to (XXXV) can be deduced.



(XXXVI)



(XXXVII)



(XXXVIII)

Related triterpenoids of the β-amyrin group, whose configurations in rings C, D, and E are clarified by the above work, are echinocystic acid (XXXVI; R = H) with a polar hydroxyl at $C_{(16)}$, maniladiol (XXXVII; R = H) with the opposite configuration at $C_{(16)}$, and glycyrrhetic acid (XXXVIII; R = H) whose $C_{(20)}$-carboxyl group can, apparently, lactonise on to $C_{(13)}$ (Ruzicka and Marxer, *Helv. Chim. Acta*, 1939, **22**, 195; Ruzicka and Jeger, *ibid.*, 1942, **25**, 775). Our experiments do *not* relate the configuration at $C_{(10)}$ (and hence elsewhere in rings C, D, and E) to that at $C_{(5)}$ and at $C_{(9)}$ and this problem, in our opinion, remains unsolved (cf. Gutmann, Jeger, and Ruzicka, *Helv. Chim. Acta*, 1951, **34**, 1154). However, there would appear to be only two possibilities which will accommodate (*a*) the reactions at $C_{(11)}$ reported above and (*b*) the fact that rings B, C, and D are fused in the more stable arrangement. These are represented in Fig. 1 (B–C *cis*, $C_{(9)}$:$C_{(14)}$ *syn*,

* It seems generally true that lithium aluminium hydride reduction of a keto-group which is *not* hindered sterically affords the equatorial hydroxyl group (see, *e.g.*, Shoppee and Summers, *J.*, 1950, 687). On the other hand, similar reduction of keto-groups which are subject to marked hindrance, *e.g.*, $C_{(11)}$ in the steroid series and $C_{(11)}$ and $C_{(19)}$ in the triterpenoid series, gives the polar hydroxyl group. Evidence for the hindrance at $C_{(11)}$ in the triterpenoid series is that attempted Wolff–Kishner reduction of (XXXI; R = Ac) at 180° gave back, after methylation and reacetylation, unchanged starting material (see Experimental).

C/D *trans*) and Fig. 2 (B/C *trans*, $C_{(9)}$:$C_{(14)}$ *anti*, C/D *trans*), which depict our alternative interpretations of the stereochemistry of oleananolic acid.*

The elegant inter-relationship of lupeol and β-amyrin through a common reaction product, β-amyrene III (XXXIX) recently reported by Ames, Halsall, and Jones (*J.*, 1951, 450) and further extended to include a conversion of betulinic acid (XL; R = R′ = H) into moradiol (XLI; R = H) (Davy, Halsall, and Jones, *J.*, 1951, 2696), demonstrates identical configurations in the lupeol and the β-amyrin series so far as positions 2, 5, 6, 9, 10, 14, and 17 are concerned and shows that $C_{(13)}$ must be configurationally the same as in morolic and oleananolic acids. Davy, Halsall, Jones, and Meakins (*J.*,

FIG. 1.

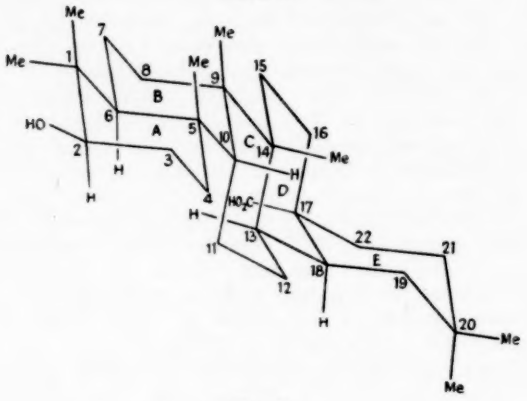
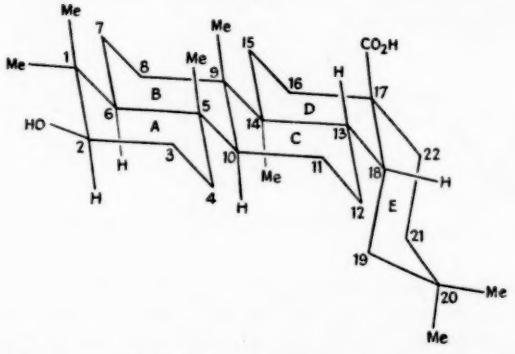


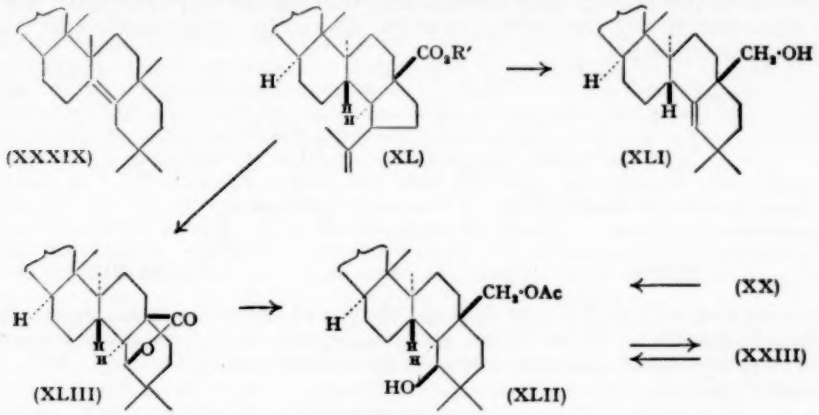
FIG. 2.



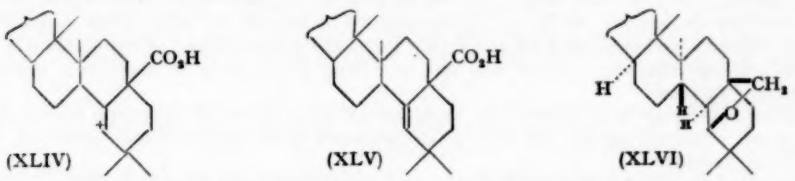
1951, 702) have recently discussed the stereochemistry at $C_{(18)}$ in the lupeol series and have concluded that the D/E ring fusion must be *trans*. Through the kindness of Professor E. R. H. Jones, F.R.S., and Dr. T. G. Halsall in informing us of their results before general publication it became possible to confirm this conclusion by the partial synthesis of the diacetate (XLII; R = Ac) by methods of stereochemical unambiguity (see Barton and Holness, *ibid.*, p. 233). Thus lithium aluminium hydride reduction of both (XX; R = Ac) and (XXIII; R = Ac), followed by reacetylation, furnished a triol diacetate (XLII; R = Ac) identical in all respects with a specimen of "betulin triol diacetate" kindly provided by Professor E. R. H. Jones and Dr. T. G. Halsall. The latter had been prepared from betulinic acid through the lactone (XLVI; R = Ac), followed by lithium aluminium hydride reduction, reactions which preserve the configuration at $C_{(18)}$ and confirm that

* It will be seen that Figs. 1 and 2 assign a definite configuration for $C_{(9)}$ relative to $C_{(5)}$. This is only a probable, not a firmly established, assignment. It is hoped to present the relevant arguments in detail in a later paper.

the unesterified hydroxyl group in (XLII; R = Ac) must be (*a*) polar in character and (*b*) on the same side of the molecule as the $C_{(17)}$-acetoxymethyl group. Chromic acid oxidation of (XLII; R = Ac) regenerated its precursor (XXIII; R = Ac).



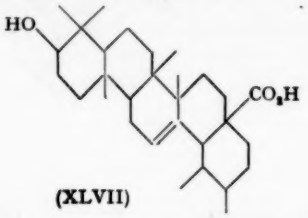
The above proof of configuration at $C_{(18)}$ in lupeol assumes that the lactone (XLIII; R = Ac) is formed from the carbonium ion (XLIV; R = Ac) *without* inversion at $C_{(18)}$. It would appear a possible criticism of this proof that the lactone formation might actually proceed through morolic acid acetate (XLV; R = Ac) (Barton and Brooks, *loc. cit.*), or that there might be an equilibrium between this acid and the lactone in a suitably acidic medium. Both possibilities were excluded in the following ways. First, treatment



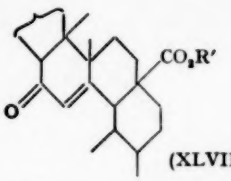
of morolic acid with hydrogen bromide in acetic acid furnished in excellent yield 18-*iso*-oleanolic acid lactone acetate (see above) which was further characterised by oxidation of the derived alcohol to the corresponding ketone. Secondly, it is well known that treatment of betulin with acetic acid containing a little sulphuric acid or with refluxing formic acid affords *allo*betulin acetate or formate respectively. The constitution of *allo*betulin has been proved (Davy, Halsall, Jones, and Meakins, *loc. cit.*) to be represented by (XLVI; R = H) by direct oxidation of its acetate to the lactone (XLIII; R = Ac). *allo*Betulin could, in principle, be formed *via* a carbonium ion [as (XLIV)] or by cyclisation of moradiol [as (XLV)]. In fact, moradiol is converted into the diacetate or diformate under the conditions, required for the production of *allo*-betulin acetate or formate. These compounds must, therefore, be formed from betulin without inversion at $C_{(18)}$.

We have not carried out extensive experiments in the α-amyrin series, but several interesting observations have been made with ursolic acid. The experiments of Ruzicka, Jeger, and their collaborators (*Helv. Chim. Acta*, 1945, **28**, 199; 1947, **30**, 140, 1294; 1949, **32**, 1075; 1950, **33**, 700, 889) have shown that the configurations at positions 2, 5, 6, 9 and, probably, 10 and 14 are the same in oleanolic and ursolic (XLVII; R = H) acids. In an effort to obtain information about the asymmetry at $C_{(18)}$ ursolic acid was treated with chloroformic hydrogen chloride. The acid was thereby equilibrated with its hitherto unknown lactone, although the equilibrium content of the lactone was only about 10%. At first, this seemed to indicate a *syn*-relation at $C_{(13)}$–$C_{(18)}$ in the lactone (cf. above).

However, reaction of methyl 11-ketoursolate acetate (XLVIII; R = Ac, R′ = Me) with either acid or base, unlike the behaviour of the corresponding 11-keto-oleanolic acid compound (see above), failed to cause isomerisation. Indeed, the ursolic acid derivative was far more stable towards these reagents than the oleanolic acid compound. We conclude either that the $C_{(18)}$ configuration is in the more stable arrangement *or* that there is no hydrogen attached to $C_{(18)}$.



(XLVII)



(XLVIII)

In connection with an *X*-ray analysis of triterpenoid compounds being undertaken in the Physics Department of this College we have prepared methyl oleanolate iodoacetate *via* the corresponding chloroacetate following the method used by Curtis (*J.*, 1950, 1017) in the lanosterol field.

Experimental

M.p.s are uncorrected. They were determined in open capillaries in the usual way unless specified to the contrary. Rotations were determined in chloroform solution. Values of $[\alpha]_D$ have been approximated to the nearest degree. Ultra-violet absorption spectra were measured in absolute ethanol solution with the Unicam S.P. 500 Spectrophotometer.

Savory and Moore's standardised alumina for chromatography was used unless specified to the contrary. Light petroleum refers to that fraction of b. p. 40—60°.

The phrase " in the usual way " implies, in general, dilution with water, extraction with ether, washing successively with aqueous potassium hydroxide (or other more suitable basic reagent), aqueous hydrochloric acid, and water, followed by evaporation of the ethereal solution *in vacuo*. Where necessary, water was removed from the residue by azeotropic distillation with benzene *in vacuo*.

Alkaline hydrolyses were effected by using several equivalents of potassium hydroxide and refluxing the reactants for 30—60 minutes in methanol or dioxan–methanol according to the solubility of the ester.

For methylations with diazomethane the acid was treated with an excess of ethereal diazomethane. After the mixture had been kept at room temperature until the evolution of nitrogen had ceased, the excess of diazomethane and the ether were removed *in vacuo* or on the steam-bath.

Oleanolic Lactone.—Purified oleanolic acid, $[\alpha]_D$ +77° (*c*, 3·26), in chloroform (25 ml.) was treated with a stream of gaseous hydrogen chloride at room temperature and then poured into water. The product was separated into acid and neutral fractions in the usual way. The neutral fraction was characterised as *oleanolic lactone* which, recrystallised from chloroform–methanol, had m. p. 278° (decomp.), $[\alpha]_D$ +11° (*c*, 6·90), +12° (*c*, 3·08) (Found: C, 77·5; H, 10·6. $C_{30}H_{48}O_2,0{\cdot}5CH_3{\cdot}OH$ requires C, 77·45; H, 10·7%). Acetylation with pyridine and acetic anhydride overnight at room temperature afforded *oleanolic lactone acetate*; recrystallised from methanol, it had m. p. 293—295°, $[\alpha]_D$ +19° (*c*, 1·83) (Found: C, 76·65; H, 9·9. $C_{32}H_{50}O_4$ requires C, 77·05; H, 10·1%). The acid fraction from the equilibration was characterised as pure oleanolic acid from its m. p., mixed m. p., and rotation (where appropriate). The results are summarised in the following Table.

Wt. of oleanolic acid taken, mg.	Duration of HCl treatment	Wt. of lactone formed, mg. (%)	Wt. of recovered acid, mg.	$[\alpha]_D$ of recovered acid
190	1 min.	51 (26%)	—	—
480	15 min.	120 (25%)	370	+76° (*c*, 1·54)
400	1 hr.	95 (24%)	300	—
450	8 hr.	108 (24%)	—	—
1330	17 hr.	320 (24%)	—	+76·5° (*c*, 1·74)
190 *	1 hr.	45 (24%)	—	+76° (*c*, 1·77)

* Of oleanolic lactone.

Methyl 11-*Keto-olean*-12 : 18-*dienolate Acetate*.—Methyl 11-keto-oleanolate acetate (280 mg.), m. p. 242—244°, $[\alpha]_D$ +83° (*c*, 6·67) (Mower, Green, and Spring, *J.*, 1944, 256), was treated in 5 ml. of " AnalaR " acetic acid at 80° with three drops of 40% hydrogen bromide–acetic acid and then with 1·6 ml. of 6% bromine–acetic acid during 15 minutes. The mixture was heated for a further 45 minutes, diluted with water, and worked up in the usual way. Filtration in benzene solution through alumina, followed by recrystallisation from methanol, gave *methyl* 11-*keto-olean*-12 : 18-*dienolate acetate*, m. p. 266—268° (decomp.), $[\alpha]_D$ +258° (*c*, 2·11) (Found : C, 75·4; H, 9·3. $C_{33}H_{48}O_5$ requires C, 75·55; H, 9·15%). When this compound (100 mg.) was heated with 20 ml. of 10% potassium hydroxide in *n*-propanol under reflux for 48·5 hours, worked up in the usual way, methylated, and then acetylated, it was recovered unchanged, m. p. 265—267°, undepressed on admixture with starting material.

Methyl ψ-11-*Keto-oleanolate Acetate*.—(a) *Acid procedure* (see Kitasato, *Acta Phytochim.*, 1934, **8**, 1). Methyl 11-keto-oleanolate acetate (1 g.) in 50% hydrobromic acid–acetic acid (20 ml.) was left for 1 week. After working up in the usual way methyl ψ-11-keto-oleanolate acetate was obtained. Purified by chromatography over alumina and recrystallisation from methanol this compound had m. p. 308—309°, $[\alpha]_D$ +84° (*c*, 1·36), $\lambda_{max.}$ 248 mμ, $\varepsilon_{max.}$ 14,100 (Found : C, 74·65; H, 9·6. Calc. for $C_{33}H_{50}O_5$: C, 75·25; H, 9·6%).

(b) *Alkaline procedure*. Methyl 11-keto-oleanolate acetate (1·17 g.) in 10% potassium hydroxide–*n*-propanol (30 ml.) was refluxed for 33 hours. Dilution with water and working up in the usual way gave an acid fraction which, after methylation, acetylation, and recrystallisation, afforded methyl ψ-11-keto-oleanolate acetate identical with that prepared by Kitasato's procedure. The latter method is to be preferred.

Methyl 18-iso*Oleanolate Acetate*.—Methyl ψ-11-keto-oleanolate acetate (220 mg.) was hydrogenated in acetic acid solution overnight using a platinum catalyst. Working up in the usual way afforded *methyl* 18-iso*oleanolate acetate* (recrystallised from chloroform–methanol), m. p. 250—252°, $[\alpha]_D$ +44° (*c*, 1·96) (Found : C, 77·2; H, 9·85. $C_{33}H_{52}O_4$ requires C, 77·3; H, 10·2%). This compound showed $\lambda_{max.}$ 199 mμ, $\varepsilon_{max.}$ 3800, ε_{215} 1100 (*c*, 0·0052), indicative of a triply substituted ethylenic linkage. In agreement, methyl oleanolate acetate itself showed $\lambda_{max.}$ 201 mμ, $\varepsilon_{max.}$ 4200, ε_{215} 1300 (*c*, 0·0091).

Methyl 18-*iso*oleanolate acetate (40 mg.) in " AnalaR " acetic acid (10 ml.) was refluxed with selenium dioxide (25 mg.) for 1 hour. The reaction product, worked up in the usual way, filtered in benzene solution through alumina, and recrystallised from chloroform–methanol, had m. p. 223—224°, $[\alpha]_D$ −128° (*c*, 0·68), $\lambda_{max.}$ 242, 252, and 261 mμ, $\varepsilon_{max.}$ 25,500, 29,100 and 18,900 respectively, and was undepressed in m. p. on admixture with an authentic specimen of methyl dehydro-oleanolate acetate, m. p. 223—224°, $[\alpha]_D$ −128° (*c*, 4·49).

18-iso*Oleanolic Acid Acetate*.—Methyl 18-*iso*oleanolate acetate (200 mg.) was heated with absolute ethanol (5 ml.) containing 200 mg. of dissolved sodium for 17 hours at 180°. The reaction product, worked up and acetylated in the usual way, gave 18-iso*oleanolic acid acetate* (recrystallised from methanol), m. p. 270—272°, $[\alpha]_D$ +50° (*c*, 2·40) (Found : C, 77·25; H, 10·6. $C_{32}H_{50}O_4$ requires C, 77·05; H, 10·1%).

18-iso*Oleanolic Lactone Acetate*.—18-*iso*Oleanolic acid acetate (80 mg.) in chloroform (10 ml.) was treated with a stream of dry hydrogen chloride for 30 minutes. Working up in the usual way gave 18-iso*oleanolic lactone acetate* (no acid fraction), m. p. 340—345° (decomp.) (recrystallised from chloroform–methanol), $[\alpha]_D$ +23° (*c*, 1·45) (Found : C, 76·95; H, 10·3. $C_{32}H_{50}O_4$ requires C, 77·05; H, 10·1%). The lactone was quantitatively recovered unchanged after 530 hours in chloroform saturated with hydrogen chloride. No trace of acid was produced. Under the same conditions oleanolic acid (60 mg.) gave the lactone (14 mg., 24%) in the usual equilibrium proportion.

Alkaline hydrolysis of the acetate lactone in the usual way gave 18-iso*oleanolic lactone* which, recrystallised from methanol, had m. p. 335—338° (decomp.), $[\alpha]_D$ +13° (*c*, 1·79) (Found : C, 77·9; H, 10·35. $C_{30}H_{48}O_3,CH_3{\cdot}OH$ requires C, 77·45; H, 10·65%).

Treatment of oleanolic acid with hydrochloric and acetic acids, or with hydrogen bromide (50%) in acetic acid, according to the directions of Winterstein and Stein (*Z. physiol. Chem.*, 1931, **199**, 64) afforded 18-*iso*oleanolic lactone acetate (previously described as " oleanolic acid lactone acetate "), m. p. 350—353° (decomp.) (from chloroform–methanol), $[\alpha]_D$ +23° (*c*, 1·20), in excellent yield. There was no depression in m. p. on admixture with authentic 18-*iso*-lactone acetate. Similar treatment of oleanolic lactone (see above) with hydrogen bromide in acetic acid likewise afforded the 18-*iso*-lactone acetate in good yield.

Lactonisation of Morolic Acid.—Morolic acid (1·0 g.) in 50% hydrogen bromide–acetic acid (20 ml.) was left for 4 days at room temperature. Dilution with water gave 18-*iso*oleanolic

lactone acetate (375 mg.) (recrystallised from chloroform–methanol), m. p. 345—350° (decomp.), $[\alpha]_D$ +23° (*c*, 1·24), +22° (*c*, 1·90). The mixed m. p. with authentic material was undepressed at 341—346° (decomp.). Hydrolysis of the acetate (110 mg.) in the usual way and oxidation of the product with chromium trioxide (25 mg.) in " AnalaR " acetic acid gave 18-iso*oleanonic lactone* (recrystallised from chloroform–methanol), m. p. 323—325° (decomp.), $[\alpha]_D$ +35° (*c*, 1·10) (Found : C, 77·8; H, 10·1. $C_{30}H_{46}O_3$,0·5CH_3·OH requires C, 77·85; H, 10·3%).

12-*Hydroxyoleanolic Acetate Lactone.*—(*a*) *Use of perhydrol–acetic acid* (Ruzicka, Hösli, and Hofmann, *Helv. Chim. Acta*, 1936, **19**, 109). Oleanolic acid acetate (1·0 g.) in acetic acid (40 ml.) was treated on the steam-bath with a mixture of acetic acid (12 ml.) and perhydrol (12 ml.). Addition of hot water (20 ml.) gave the crystalline 12-hydroxyoleanolic acetate lactone (530 mg.), further purified by recrystallisation from chloroform–methanol. For physical data see the Table below.

(*b*) *Use of perbenzoic acid* (Picard and Spring, *J.*, 1940, 1387). Oleanolic acid acetate (670 mg.) in 40 ml. of 0·15 N-perbenzoic acid solution in chloroform was left for 17 days at 0°. The uptake of perbenzoic acid was 115% of theory. Working up in the usual way gave the 12-hydroxy-lactone with the properties recorded below.

Method of prep. of 12-hydroxy-oleanolic acetate lactone.	12-Hydroxy-lactone, m. p.*†	$[\alpha]_D$	12-Acetoxy-lactone, m. p.*	$[\alpha]_D$
(*a*) Perhydrol–acetic acid	296—297°; then 317—327° (decomp.)	+47° (*c*, 2·52)	275–285° (decomp.)	+62° (*c*, 0·98)
(*b*) Perbenzoic acid	296—297°; then 327—330° (decomp.)	+44° (*c*, 1·71)	274—282° (decomp.)	+60° (*c*, 1·07)
(*c*) Potassium permanganate	293°; then 322—324° (decomp.)	+42° (*c*, 1·23)	272—282° (decomp.)	+59° (*c*, 1·75

* The appropriate mixed m.p.s showed no depression.
† Taken in evacuated capillaries.

(*c*) *Use of potassium permanganate* (Aumüller, Wedekind, and Schicke, *Annalen*, 1935, **517**, 211). Oleanolic acid acetate (2·5 g.) in " AnalaR " acetone (200 ml.) and 2N-aqueous sulphuric acid (12·5 ml.) was oxidised at reflux temperature by the addition of a saturated solution of potassium permanganate (5 g.) in acetone. Working up in the usual way gave the 12-hydroxy-lactone (800 mg.) (see Table).

12-Hydroxyoleanolic acetate lactone (190 mg.), treated with pyridine (8 ml.) and acetic anhydride (3 ml.) at room temperature overnight, was recovered unchanged. However, heating at 100° for 1·5 hours with the same reagent gave the diacetate, recrystallised from chloroform–methanol. In a further experiment the heating time was ½ hour. These conditions also produced complete acetylation.

Methyl Olean-10-enolate Acetate.—Methyl 12-keto-olean-10-enolate acetate, m. p. 202—204°, $[\alpha]_D$ +53° (*c*, 2·10), +53° (*c*, 1·82), $\lambda_{max.}$ 247 mμ, $\varepsilon_{max.}$ 10,600, was prepared both by Picard and Spring's method (*J.*, 1939, 1045) and by Ruzicka, Jeger, and Winter's (*Helv. Chim. Acta*, 1943, **26**, 265). Although the latter procedure is more expeditious the former method gave better yields.

Methyl 12-keto-olean-10-enolate acetate (1·07 g.) was hydrogenated in " AnalaR " acetic acid (50 ml.) at room temperature over a platinum catalyst for 15 hours. Worked up in the usual way the product of the reaction was chromatographed over alumina. Benzene–light petroleum (1 : 9) removed only a trace of oil, but a 1 : 3 mixture was effective. An early fraction gave, on recrystallisation from methanol, long needles of *methyl olean-10-enolate acetate*, m. p. 190—192°, $[\alpha]_D$ +52° (*c*, 1·27), $\lambda_{max.}$ 199 mμ, $\varepsilon_{max.}$ 3500, ε_{215} 350 (*c*, 0·0093) (Found : C, 77·15; H, 10·1. $C_{33}H_{52}O_4$ requires C, 77·3; H, 10·2%); there was no indication of any selective absorption at 282 mμ. Final elution with benzene alone gave material which, recrystallised from chloroform–methanol, afforded methyl olean-10 : 12-dienolate acetate, m. p. 207—209°, $[\alpha]_D$ +244° (*c*, 2·14), +242° (*c*, 1·25), $\lambda_{max.}$ 282 mμ, $\varepsilon_{max.}$ 9800 (Found : C, 77·75; H, 9·65. Calc. for $C_{33}H_{50}O_4$: C, 77·6; H, 9·85%).

Methyl olean-10-enolate acetate was more conveniently prepared in the following way. Methyl 12-keto-olean-10-enolate acetate (2·0 g.) was heated with sodium (1·7 g.) dissolved in absolute ethanol (15 ml.) and 95% hydrazine (8 ml.) in a sealed tube at 180° for 18 hours. After working up in the usual way the acid product was methylated, acetylated, and then chromatographed over alumina (9 fractions). Elution with 40 : 60 and 50 : 50 benzene–light petroleum gave, after recrystallisation from methanol, 500 mg. of the required acetate methyl ester.

Reduction of 11-*Keto-oleanolic Acid Acetate.*—11-Keto-oleanolic acid acetate (450 mg.) in

ethanol (18 ml.) was refluxed (oil-bath) and sodium (1·3 g.) added during ½ hour. The reaction product was worked up in the usual way, methylated, and acetylated with refluxing acetic anhydride and sodium acetate. Chromatography over alumina gave methyl olean-10 : 12-dienolate acetate; eluted with light petroleum and crystallised from chloroform-methanol, it had $[\alpha]_D$ +243° (*c*, 0·82) and m. p. 208—210° alone or mixed with the diene, m. p. 208—210°, obtained by the catalytic hydrogenation reported above. Kitsato (see Elsevier's " Encyclopaedia of Organic Chemistry," Vol. XIV, p. 546) recorded m. p. 225—228°, $[\alpha]_D$ +230°, for this compound; we have not been able to duplicate this high m. p.

Methyl 11-*Keto-oleananolate Acetate*.—Methyl olean-10-enolate acetate (450 mg.) in " AnalaR " acetic acid (45 ml.) was treated with a mixture of perhydrol (7 ml.) and acetic acid (7 ml.) added during 2 hours whilst being heated on the steam-bath. Worked up in the usual way the reaction product had m. p. 260—272°. Recrystallisation from chloroform–methanol furnished *methyl* 11-*keto-oleananolate acetate*, m. p. 272—274°, $[\alpha]_D$ +3° (*c*, 3·05), +4° (*c*, 1·34), $\lambda_{max.}$ 292 mμ, $\epsilon_{max.}$ 30 (Found: C, 74·8; H, 9·8. $C_{33}H_{52}O_5$ requires C, 74·95; H, 9·9%). This compound also crystallises in a different form, m. p. 255—257°.

When methyl 11-keto-oleananolate acetate (75 mg.) was heated with sodium (100 mg.) dissolved in ethanol (2 ml.) and 95% hydrazine (1 ml.) at 180° for 18 hours, the product, after remethylation and reacetylation, was characterised as starting material by m. p. and mixed m. p.

2 : 28-*Diacetoxyoleanan*-11-*ol*.—Lithium aluminium hydride (450 mg.) was refluxed for 5 minutes in sodium-dried ether (10 ml.), and the solution rapidly filtered into a solution of methyl 11-keto-oleananolate acetate (100 mg.) in dry ether (20 ml.). After 1 hour's refluxing the excess of lithium aluminium hydride was destroyed by addition of a few drops of ethyl acetate. The reaction mixture was then diluted with water, acidified with dilute sulphuric acid, and worked up in the usual way. Recrystallisation of the product from aqueous methanol furnished fine short needles of oleanane-2 : 11 : 28-triol, softening at 120°, clearing of bubbles at 127°, and mobile at 145°, $[\alpha]_D$ +19° (*c*, 1·20). The triol was acetylated overnight at room temperature, with pyridine and acetic anhydride. Working up in the usual way gave a crude product, m. p. 241—245°; recrystallised from methanol containing a few drops of chloroform it afforded 2 : 28-*diacetoxyoleanan*-11-*ol*, m. p. 267—270°, $[\alpha]_D$ +31° (*c*, 1·50) (Found: C, 74·85; H, 9·9. $C_{34}H_{56}O_5$ requires C, 74·95; H, 10·35%).

Conversion into the triacetate was effected in the following way. The triol diacetate (25 mg.) was heated with acetic anhydride (0·5 ml.) and pyridine (3 ml.) at 100° for 5 hours. Working up the dark product in the usual way, and filtration in benzene solution through alumina, gave 2 : 11 : 28-*triacetoxyoleanane* (20 mg.) which, recrystallised from methanol, had m. p. 275—277°, $[\alpha]_D$ +55° (*c*, 0·87) (Found: C, 73·35; H, 9·8. $C_{36}H_{58}O_6$ requires C, 73·7; H, 9·95%). In comparable experiments 75 mg. of the diacetate gave 10 mg. of the triacetate (1 hour's heating) and 23 mg. of the diacetate afforded 8 mg. of the triacetate (2 hours' heating).

2 : 28-*Diacetoxyoleanan*-11-*one*.—2 : 28-Diacetoxyoleanan-11-ol (100 mg.) in " AnalaR " acetic acid (3 ml.) was oxidised with chromium trioxide (70 mg.), and the solution left overnight at room temperature. Working up in the usual way, and filtration in benzene solution through alumina, gave 2 : 28-*diacetoxyoleanan*-11-*one* which, recrystallised from methanol, had m. p. 260—262°, $[\alpha]_D$ +6° (*c*, 1·41) (Found: C, 74·55; H, 9·9. $C_{34}H_{54}O_5$ requires C, 75·25; H, 10·0%). There was a marked depression in m. p. on admixture with starting material.

2 : 28-*Diacetoxyolean*-10-*ene*.—(a) *From methyl olean*-10-*enolate acetate*. The methyl ester acetate (50 mg.) was refluxed for ½ hour with a filtered solution of lithium aluminium hydride (300 mg.) in dry ether (50 ml.). Working up as above, and acetylation of the product with pyridine and acetic anhydride at 100° for ½ hour, afforded 2 : 28-*diacetoxyolean*-10-*ene* (recrystallised from methanol), m. p. 204—205°, $[\alpha]_D$ +59° (*c*, 1·03) (Found: C, 77·15; H, 10·25. $C_{34}H_{54}O_4$ requires C, 77·5; H, 10·35%).

(b) *By dehydration of* 2 : 28-*diacetoxyoleanan*-11-*ol*. The triol diacetate (25 mg.) in dry pyridine (3 ml.) was treated with redistilled phosphorus oxychloride (0·3 ml.), and the solution heated for 3 hours at 100°. Dilution with water, extraction with ether, and working up in the usual way, furnished 2 : 28-diacetoxyolean-10-ene; recrystallised from methanol, this had m. p. 200—202°, $[\alpha]_D$ +56° (*c*, 0·99). The mixed m. p. with authentic material (m. p. 204—205°; see above) was 201—203°.

Methyl 19-*Keto-oleananolate Acetate*.—Methyl dihydrosiaresinolate acetate (110 mg.), prepared as described before (Barton, Brooks, and Holness, *J.*, 1951, 277) in " AnalaR " acetic acid (20 ml.) was treated with chromium trioxide (25 mg.) in 95% " AnalaR " acetic acid (25 ml.) and left overnight. Working up in the usual way and recrystallisation from methanol

afforded *methyl* 19-*keto-oleananolate acetate*, m. p. 228—230°, $[\alpha]_D$ +46° (*c*, 1·83) (Found : C, 75·1; H, 9·7. $C_{33}H_{52}O_5$ requires C, 74·95; H, 9·95%).

Methyl 19-*Keto*-18-iso*oleananolate Acetate*.—Methyl 19-keto-oleananolate acetate (80 mg.) was refluxed for 1 hour with 10% methanolic potassium hydroxide (20 ml.). Working up in the usual way gave *methyl* 19-*keto*-18-iso*oleananolate*, m. p. 274—275° (from methanol), $[\alpha]_D$ +52° (*c*, 1·20) (Found : C, 76·85; H, 10·7. $C_{31}H_{50}O_4$ requires C, 76·5; H, 10·35%). Acetylation by pyridine–acetic anhydride overnight and working up in the usual way afforded *methyl* 19-*keto*-18-iso*oleananolate acetate*, m. p. 285—287° (from methanol), $[\alpha]_D$ +53° (*c*, 1·40) (Found : C, 74·65; H, 9·85. $C_{33}H_{52}O_5$ requires C, 74·95; H, 9·95%).

2 : 28-*Diacetoxyoleanan*-19-*ol*.—Methyl dihydrosiaresinolate acetate (250 mg.) in dry ether (40 ml.) was refluxed with a filtered solution of lithium aluminium hydride (750 mg.) in dry ether (30 ml.) for 2·5 hours. Decomposition of the excess of lithium aluminium hydride with excess of ethyl acetate, acidification with dilute sulphuric acid, working up in the usual way, and finally acetylation (pyridine–acetic anhydride at 100° for 30 minutes) afforded 2 : 28-*diacetoxyoleanan*-19-*ol* which, recrystallised from chloroform–methanol, had m. p. 255—256°, $[\alpha]_D$ +23° (*c*, 1·69) (Found : C, 74·8; H, 10·35. $C_{34}H_{56}O_5$ requires C, 75·0; H, 10·35%).

2 : 28-*Diacetoxyoleanan*-19-*one*.—2 : 28-Diacetoxyoleanan-19-ol (60 mg.) in " AnalaR " acetic acid (10 ml.) was left overnight at room temperature with chromium trioxide (15 mg.) in 95% acetic acid (2 ml.). Working up in the usual way and recrystallisation from chloroform–methanol furnished 2 : 28-*diacetoxyoleanan*-19-*one*, m. p. 246—248°, $[\alpha]_D$ +50° (*c*, 1·00) (Found : C, 75·15; H, 10·15. $C_{34}H_{54}O_5$ requires C, 75·25; H, 10·05%).

This compound (50 mg.) was refluxed with 30% methanolic potassium hydroxide (20 ml.) for 2 hours. After working up in the usual way, acetylation (pyridine–acetic anhydride overnight at room temperature) and repeated recrystallisation from methanol gave 2 : 28-*diacetoxy*-18-iso*oleanan*-19-*one*, m. p. 214—216° (Found : C, 75·25; H, 9·85. $C_{34}H_{54}O_5$ requires C, 75·25; H, 10·05%).

2 : 28-*Diacetoxy*-18-iso*oleanan*-19-*ol*.—Methyl 19-keto-18-*iso*oleananolate acetate (240 mg.) (see above) in dry ether (30 ml.) was refluxed with a filtered solution of lithium aluminium hydride (600 mg.) in dry ether (20 ml.) for 1·5 hours. Working up in the usual way and acetylation with acetic anhydride–pyridine for 3 hours at room temperature furnished 2 : 28-diacetoxy-18-*iso*oleanan-19-ol (" betulin triol diacetate ") which, recrystallised from chloroform–methanol, had m. p. 239—241°, $[\alpha]_D$ +25° (*c*, 0·61). This gave no depression in m. p. on admixture with an authentic specimen of " betulin triol diacetate," m. p. 240—242°, $[\alpha]_D$ +22° (*c*, 0·78), kindly supplied by Professor E. R. H. Jones, F.R.S., and Dr. T. G. Halsall.

Oxidation of 2 : 28-diacetoxy-18-*iso*oleanan-19-ol with chromium trioxide in aqueous acetic acid in the usual way, followed by chromatography (11 fractions), gave 2 : 28-diacetoxy-18-*iso*-oleanan-19-one (recrystallised from methanol), m. p. 216—218°, $[\alpha]_D$ +41° (*c*, 1·13). There was no depression in m. p. on admixture with (*a*) the same compound (m. p. 214—216°) prepared by the alternative route given above or (*b*) the ketone of the same m. p. and $[\alpha]_D$ +37° (*c*, 0·90) prepared by Davy, Halsall, Jones, and Meakins (*loc. cit.*) by oxidation of " betulin triol diacetate." We thank Professor Jones and Dr. Halsall for carrying out the mixed m. p. determination.

Effect of Alkali on Methyl 12-*Keto-oleananolate Acetate*.—The methyl ester acetate (150 mg.) was refluxed with 10% (w/v) methanolic potassium hydroxide (10 ml.) for 1 hour. Worked up in the usual way the product was acetylated (pyridine–acetic anhydride overnight at room temperature). Recrystallisation gave unchanged starting material, m. p. 191—193° alone or mixed with authentic methyl ester acetate of the same m. p. This experiment shows that $C_{(13)}$ is not inverted by the action of potassium hydroxide.

Derivatives of Moradiol.—Moradiol (Barton and Brooks, *J.*, 1951, 257) (100 mg.) in glacial acetic acid (10 ml.) was treated with 2 drops of concentrated sulphuric acid and heated for 3 hours on the steam-bath. Working up in the usual way and recrystallisation from chloroform–methanol furnished moradiol diacetate, m. p. 273° alone or mixed with authentic material of the same m. p.

Under the same conditions betulin was converted almost quantitatively into *allo*betulin acetate, m. p. 280—282°.

Moradiol (250 mg.) was refluxed with 100% formic acid (5 ml.). A thick paste of crystals was rapidly deposited. Working up in the usual way and recrystallisation from chloroform–methanol furnished *moradiol diformate*, m. p. 284—286°, $[\alpha]_D$ +22° (*c*, 1·57) (Found : C, 77·55; H, 10·2. $C_{32}H_{50}O_4$ requires C, 77·05; H, 10·1%). Alkaline hydrolysis gave back moradiol (mixed m. p.).

Betulin (1 g.), treated with 100% formic acid (9 ml.) in the same way, gave *allo*betulin formate, m. p. 310—312° (decomp.) (in an evacuated capillary) (cf. Schulze and Pieroh, *Ber.*, 1922, **55**, 2332).

Moradiol Oxide.—Moradiol diacetate oxide (Barton and Brooks, *loc. cit.*) (200 mg.) was refluxed with 10% (w/v) methanolic potassium hydroxide (20 ml.) for 1 hour. Working up in the usual way and crystallisation from aqueous methanol gave *moradiol oxide*, m. p. 205—207° (decomp.), $[\alpha]_D$ +21° (*c*, 4·00) (Found: C, 78·2; H, 11·1. $C_{30}H_{50}O_3$ requires C, 78·5; H, 11·0%). Acetylation with pyridine–acetic anhydride at room temperature overnight gave back moradiol diacetate oxide, m. p. 255—256° alone or mixed with an authentic specimen of the same m. p.

Ursolic Lactone.—Ursolic acid (315 mg.), m. p. 282—283°, $[\alpha]_D$ +72° (*c*, 1·45), extracted from bearberry leaves, was treated in chloroform with hydrogen chloride, as for oleanolic acid (see above). Separation of the equilibrated product furnished a neutral fraction (28 mg., 9%), recrystallised from methanol to give *ursolic lactone*, m. p. 256—258°, $[\alpha]_D$ +4° (*c*, 1·43), no absorption of $E^{1\%}_{1\,cm.} > 1$ in the 195—350-mμ region (Found: C, 79·5; H, 10·2. $C_{30}H_{48}O_3$ requires C, 78·9; H, 10·6%). The acid fraction was characterised as unchanged ursolic acid. A second experiment showed 12% of lactone in the equilibrium mixture.

Acetylation of the lactone with pyridine–acetic anhydride overnight at room temperature gave the corresponding acetate, m. p. 252—254° (from methanol), $[\alpha]_D$ +14° (*c*, 1·42), +13° (*c*, 0·76) (Found: C, 77·85; H, 10·35. $C_{32}H_{50}O_4$ requires C, 77·05; H, 10·0%).

Experiments with Methyl 11-Ketoursolate Acetate.—Methyl 11-ketoursolate acetate (250 mg.) m. p. 240—242°, $[\alpha]_D$ +81° (*c*, 2·12), $\lambda_{max.}$ 249 mμ, $\varepsilon_{max.}$ 12 400, prepared according to the directions of Ewen and Spring (*J.*, 1943, 523), was treated with 20% ethanolic potassium hydroxide (5 ml.) at 180° for 24 hours. Working up in the usual way, methylation, reacetylation, and recrystallisation from methanol, gave unchanged starting material (190 mg.), m. p. 240—242° (mixed m. p.).

In a comparable experiment 1·5 g. of the ketone was kept with 50% hydrogen bromide–acetic acid (20 ml.) at room temperature for 7 days. Working up in the usual way, filtration in benzene solution through alumina, and recrystallisation from methanol gave back unchanged starting material (740 mg.), m. p. and mixed m. p. 236—239°, $[\alpha]_D$ +81° (*c*, 0·95).

Methyl Oleanolate Chloroacetate [with G. F. Laws].—A solution of methyl oleanolate (850 mg.) in dry pyridine (25 ml.) was cooled to −30° and chloroacetyl chloride (2·6 g., 1·75 ml.) added dropwise with shaking. The solution was left without further cooling for ½ hour and then worked up in the usual way. After filtration through alumina in benzene solution and recrystallisation from chloroform–methanol, *methyl oleanolate chloroacetate* was obtained, having m. p. 190—191° (Found: Cl, 6·8. $C_{33}H_{51}O_4Cl$ requires Cl, 6·5%).

Methyl Oleanolate Iodoacetate [with G. F. Laws).—Methyl oleanolate chloroacetate (350 mg.) in acetone (25 ml.) was refluxed with powdered potassium iodide (1 g.) for 3 hours. Working up in the usual way, followed by filtration through alumina in benzene solution and crystallisation from chloroform–methanol, afforded fine white needles of *methyl oleanolate iodoacetate*, m. p. 110° (decomp.) (Found: I, 19·8. $C_{33}H_{51}O_4I$ requires I, 19·9%).

2 : 28-*Diacetoxyolean*-11 : 13(18)-*diene.*—A filtered solution of lithium aluminium hydride (200 mg.) in dry ether (20 ml.) was added to methyl dehydro-oleanolate acetate (100 mg.) in dry ether (10 ml.). The mixture was refluxed for 0·5 hour, worked up in the usual way, and acetylated with acetic anhydride (1 ml.) and pyridine (3 ml.) overnight at room temperature. Working up in the usual way afforded 2 : 28-*diacetoxyolean*-11 : 13(18)-*diene* (dehydroerythrodiol diacetate) which, recrystallised from methanol, had m. p. 214—215°, $[\alpha]_D$ −87° (*c*, 1·39), $\lambda_{max.}$ 240, 250, and 260 mμ, $\varepsilon_{max.}$ 25,000, 29,000, and 19,500 respectively (Found: C, 77·55; H, 10·05. $C_{34}H_{52}O_4$ requires C, 77·8; H, 10·0%). This was also prepared by reduction of methyl 11-keto-oleanolate acetate (250 mg.) in dry ether (30 ml.) with lithium aluminium hydride (500 mg.) in dry ether (30 ml.), refluxing for 0·5 hour. Working up in the usual way, and acetylation by refluxing with acetic anhydride (10 ml.) for 2 hours, gave the same diacetate, m. p. and mixed m. p. 215—216°.

β-*Amyranonyl Benzoate.*—β-Amyrin benzoate (2 g.) in acetic acid (150 ml.) was heated on the steam-bath and treated dropwise during 0·5 hour with a mixture of acetic acid (40 ml.) and perhydrol (40 ml.). The resulting solution was kept at 100° for 1·5 hours. Hot water was then added until turbidity appeared. After cooling to room temperature, the crystals were filtered off and recrystallised from chloroform–methanol, to give β-amyranonyl benzoate, m. p. 252—254°, $[\alpha]_D$+1 2° (*c*, 1·47) (Found: C, 80·55; H, 9·85. Calc. for $C_{37}H_{54}O_3$: C, 81·25; H, 9·95%) (cf. Simpson, *J.*, 1940, 235). That this was a ketone and not an oxide

was proved in two ways. First, heating the benzoate (50 mg.) in acetic acid (10 ml.) and concentrated hydrochloric acid (5 ml.) on the steam-bath for 15 minutes gave the starting material unchanged (m. p. and mixed m. p.). Secondly, alkaline hydrolysis of the benzoate (50 mg.), followed by acetylation, gave β-amyranonyl acetate, m. p. 293—295°, $[\alpha]_D$ −11° (*c*, 1·88). Spring (*J.*, 1933, 1345) gives m. p. 291—292° for this compound.

Reduction of β-Amyranonyl Benzoate.—β-Amyranonyl benzoate (500 mg) in *n*-propyl alcohol (20 ml.) was heated at 130° (oil-bath temp.) and treated during 1 hour with a large excess of sodium (2 g.), the bath temperature being gradually raised to 180°. A further 10 ml. of *n*-propyl alcohol was added, followed by more sodium (0·5 g.). The excess of sodium was destroyed by addition of more *n*-propyl alcohol, and the mixture was then worked up in the usual way. Recrystallisation from methanol gave *β-amyrane-2 : 12-diol*, m. p. 207—208°, $[\alpha]_D$ +6° (*c*, 1·40), +6° (*c*, 1·29) (Found : C, 81·05; H, 11·4. $C_{30}H_{52}O_2$ requires C, 81·0; H, 11·8%). The diol (25 mg.) in pyridine (2 ml.) and acetic anhydride (1 ml.) was left overnight at room temperature. Working up in the usual way and recrystallisation from aqueous methanol furnished *2 : 12-diacetoxy-β-amyrane*, m. p. 194—195°, $[\alpha]_D$ +11° (*c*, 0·97), +12° (*c*, 0·55) (Found : C, 77·5; H, 10·65. $C_{34}H_{56}O_4$ requires C, 77·3; H, 10·65%). The acetylation was also accomplished more expeditiously by acetic anhydride and pyridine on the steam-bath (1 hour).

These compounds, with equatorial hydroxyl groups at both $C_{(2)}$ and $C_{(12)}$, were reported previously by Picard, Sharples, and Spring (*J.*, 1939, 1045) to have the following constants : β-amyrane-2 : 12-diol, m. p. 216—217°, $[\alpha]_D$ +97°; its derived diacetate, m. p. 183—184°, $[\alpha]_D$ +42°. At the present time we are unable to explain this discrepancy. As further evidence for the correctness of the physical constants now reported, β-amyranonyl benzoate (see above) was hydrolysed to β-amyranonol, which was then reduced with sodium and *n*-propyl alcohol, as described above for the benzoate. The product was the β-amyrane-2 : 12-diol, m. p. 204—206°, $[\alpha]_D$ +8° (*c*, 1·52), identical (mixed m. p.) with the diol prepared by reduction of the benzoate (see above).

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19. *The Chemistry of Extractives from Hardwoods. Part V.* The Isolation of 3 : 7 : 4′-Trimethylquercetin (Ayanin) from the Heartwood of* Distemonanthus Benthamianus.

By F. E. King, T. J. King, and K. Sellars.

From the heartwood of the tree *Distemonanthus Benthamianus* a mixture of yellow colouring matters has been extracted. One of the constituents (ayanin) has been shown by degradation and synthesis to be 5 : 3′-dihydroxy-3 : 7 : 4′-trimethoxyflavone, a hitherto unknown derivative of quercetin.

The West African tree, *Distemonanthus Benthamianus*, is the source of a commercial timber known variously as ayan, movingui, or Nigerian satinwood. The heartwood is pale to bright yellow with a lustrous surface, and being moderately durable and termite-resistant it finds many applications in furniture-making and joinery. It is reported to have acid-resisting properties, and also to stain in contact with sodium carbonate, but these observations do not appear to have been followed by any chemical examination of the timber. This communication presents the preliminary results of an investigation of the material extracted by means of organic solvents, which show that the heartwood contains a mixture of yellow colouring matters believed from their general properties to belong to the flavone series. Only one of the component compounds has so far been

* Part IV, *J.*, 1951, 569.

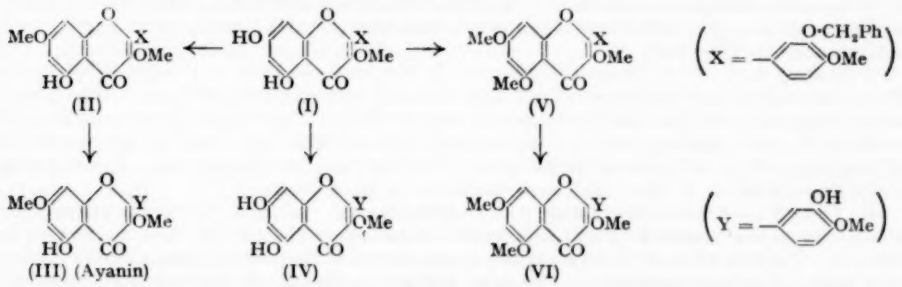
isolated in quantity sufficient to allow of its complete recognition; work on the accompanying pigments is continuing.

The identified constituent—termed ayanin in reference to the name commonly applied to the wood—is a hitherto unknown trimethyl ether of quercetin, namely, 5 : 3′-dihydroxy-3 : 7 : 4′-trimethoxyflavone. It was obtained from the heartwood by the usual sequence of extractions with boiling light petroleum, ether, and chloroform. The new colouring matter was present in all three solvent extracts, the combined yields being approximately 0·1% of the timber.

The molecular formula, $C_{18}H_{16}O_7$, for ayanin includes three methoxyl groups (Zeisel). The phenolic character of the pigment was evident from its solubility in aqueous sodium carbonate and hydroxide, but not in sodium hydrogen carbonate, to give dark orange solutions, and the presence of two hydroxyl groups was shown by the preparation of a diacetate. Indications of the nuclear structure were afforded by crimson colour reactions with magnesium and hydrochloric acid and with sodium amalgam followed by acid, denoting a flavonol nucleus with a blocked, *e.g.*, methylated, 3-hydroxyl group (Asahina and Inubuse, *Ber.*, 1928, **61**, 1646; 1929, **62**, 1256; Briggs and Locker, *J.*, 1949, 2158). The ultra-violet light absorption of ayanin solutions resembles typical flavonol spectra except for the absence of the maximum at 3100 Å characteristic of the free hydroxyl in the 3-position (Skarzynski, *Biochem. Z.*, 1939, **301**, 150), thus supporting the conclusions drawn from the colour reactions.

Evidence of the flavonol structure and, incidentally, of the orientation of the five substituent oxygen atoms was also found in the hydrolysis of ayanin with boiling hydriodic acid to a pentahydroxyflavone which gave an acetate apparently identical with pentaacetylquercetin. The relation to quercetin was confirmed by methylation experiments: either a tetramethoxy-compound, or under more vigorous conditions, a pentamethoxyflavone resulted, and the identity of these products was conclusively established by comparison with the corresponding derivatives prepared from an authentic specimen of quercetin. Moreover, from its reluctance to undergo complete methylation, which is, in general, a property of all 5-hydroxyflavones, etc., it was apparent that ayanin contained a free phenolic group at the 5-position.

The distribution of the remaining substituents finally became apparent as the result of the degradation of ayanin by ozonolysis. Alkaline hydrolysis of the product, presumably an aroylated *o*-hydroxybenzoylformic ester, led to the isolation of *iso*vanillic acid. This acid was also formed by the hydrolysis of ayanin with aqueous-alcoholic potassium hydroxide. The definite location of two further substituents in this way, combined with the information already gained regarding the nature of the groups at the 3- and the 5-position, completely determined the constitution of ayanin which was thereby identified as 5 : 3′-dihydroxy-3 : 7 : 4′-trimethoxyflavone (III).



The conclusions thus drawn from analytical data have been confirmed by a synthesis based on the general method of Allan and Robinson (*J.*, 1924, 2192). The necessary flavonol intermediate, 3′-benzyloxy-5 : 7-dihydroxy-3 : 4′-dimethoxyflavone (I), was prepared in excellent yield from ω-methoxyphloracetophenone and a mixture of the anhydride and sodium salt of benzyl*iso*vanillic acid, the fusion being carried out under

reduced pressure. Treatment in acetone solution with potassium carbonate and a limited amount of methyl sulphate caused methylation only of the 7-hydroxy group, giving 3′-benzyloxy-5-hydroxy-3 : 7 : 4′-trimethoxyflavone (II). Hydrolysis of the benzyl group with mixed hydrochloric and acetic acids completed the synthesis, the resulting dihydroxy-trimethoxyflavone (III) being identical with ayanin, as were also the respective diacetates. The availability of the intermediate (I) was utilised in the synthesis of two further new methyl ethers of quercetin. Of these, 5 : 7 : 3′-trihydroxy-3 : 4′-dimethoxyflavone (IV) was prepared simply by acid hydrolysis of the benzyl ether (I). To obtain 3′-hydroxy-3 : 5 : 7 : 4′-tetramethoxyflavone (VI), the parent compound (I) was first alkylated with excess of methyl sulphate thus forming the benzyl tetramethyl ether (V) from which the benzyl group was then removed by mild hydrolysis.

Experimental

Ayanin.—The ayan heartwood used in these experiments was supplied by the Forest Products Research Laboratory, Princes Risborough, through the kindness of Dr. W. P. K. Findlay. The powdered wood (2 kg.) was first extracted with boiling light petroleum (b. p. 40—60°) and, of the material thus obtained (2·1 g.), the small alkali-soluble portion (0·05 g.) was subsequently identified as ayanin. Boiling ether then removed a brownish-yellow friable solid (28·2 g.) which was heated with methanol (200 c.c.) under reflux for 1 hour. When the insoluble matter (0·4 g.) (A) had been removed, the solution was concentrated to 50 c.c. and left at room temperature for 1—2 weeks. The crystalline deposit (3 g.) which had then formed consisted of crude ayanin. When it had been collected, a further product (5 g.) (B) was obtained by concentrating the filtrate to 35 c.c. and setting it aside for several days.

The chloroform extract also consisted of a brittle light brown substance (23·1 g.) which was likewise digested with boiling methanol (100 c.c.). The residue (1 g.) (C) was collected and, after the filtrate had been concentrated to 50 c.c. and set aside for some time, a further quantity (3·7 g.) of impure ayanin was obtained. By repeated crystallisation of the combined crude fractions, pure *ayanin* (2 g.) was obtained as orange-yellow plates, m. p. 172—173° [Found, in the material dried at 110° in a vacuum: C, 62·6; H, 4·7; OMe, 27·5. $C_{15}H_7O_4(OMe)_3$ requires C, 62·8; H, 4·6; OMe, 27·0%]. Light absorption in methanol: maxima at 2540 (log ε = 3·93), 2710 (log ε = 3·93), and 3380 Å (log ε = 3·88). Ayanin is sparingly soluble in ether, readily soluble in acetone, chloroform, and the simple alcohols. It dissolved in concentrated hydrochloric acid to a bright yellow solution from which it was precipitated by water. In alcoholic solution it gave a brownish-green colour with ferric chloride. Acetylation of ayanin with acetic anhydride–pyridine or acetic anhydride–sodium acetate afforded the *diacetyl* derivative which separated from methanol in colourless leaflets, m. p. 176—177° (Found, in a specimen dried at 100° in a vacuum: C, 60·8; H, 4·6; OMe, 21·7. $C_{22}H_{20}O_9.\frac{1}{2}H_2O$ requires C, 60·4; H, 4·8; OMe, 21·3%). The investigation of products A, B, and C is in progress.

Demethylation of Ayanin.—When the solution obtained by heating ayanin (0·1 g.) with hydriodic acid (6 c.c.; *d* 1·7) and phenol (1·5 g.) at 160° for 4 hours was poured into water, a precipitate of quercetin was obtained. This was collected and acetylated with acetic anhydride–sodium acetate to give penta-acetylquercetin which crystallised from ethanol as leaflets, m. p. and mixed m. p. 193—194°.

5-Hydroxy-3 : 7 : 3′ : 4′-tetramethoxyflavone (O-*Monomethylayanin*).—A solution of ayanin (0·2 g.) in methanol (4 c.c.) was treated with excess of ethereal diazomethane. After 2 days, the solution was evaporated and the gummy residue triturated with aqueous sodium hydroxide until solid. The product (0·15 g.) crystallised from methanol in long flat lemon-coloured leaflets, m. p. 158—159°, alone or mixed with 3 : 7 : 3′ : 4′-tetramethylquercetin. The respective acetyl derivatives, m. p. 168—169°, were also shown to be identical.

3 : 5 : 7 : 3′ : 4-Pentamethoxyflavone (OO-*Dimethylayanin*).—Ayanin (0·2 g.) was heated under reflux with methyl sulphate (1 c.c.) and potassium carbonate (2 g.) in dry acetone (30 c.c.) for 8 hours. The filtered acetone solution was evaporated and the yellow gummy residue stirred with aqueous sodium hydroxide. The solid residue, repeatedly crystallised from methanol, gave colourless plates having m. p. 152·5°, which was not depressed by pentamethylquercetin (Found, in a specimen dried at 100° in a vacuum: C, 64·7; H, 5·4; OMe, 40·6. Calc. for $C_{20}H_{20}O_7$: C, 64·5; H, 5·4; OMe, 41·4%).

Degradation of Ayanin.—(i) Diacetylayanin (0·53 g.) was dissolved in ethyl acetate (45 c.c.), and the solution treated at 0° with excess of ozonised oxygen. When the solvent had been removed at low temperature in a vacuum, the gummy residue was treated with water (100 c.c.)

on a steam-bath. The insoluble product was collected and hydrolysed with warm 2N-sodium hydroxide (5 c.c.) in an atmosphere of hydrogen. Acidification precipitated *iso*vanillic acid (0·03 g.) which after crystallisation from methanol (charcoal) formed colourless plates, m. p. alone or mixed with an authentic specimen 252°. Acetylation gave acetyl*iso*vanillic acid in needles, m. p. and mixed m. p. 214—215° (Found: C, 57·1; H, 4·8. Calc. for $C_{16}H_{10}O_5$: C, 57·1; H, 5·2%). By methylating the acid with diazomethane and distilling the product an oil was obtained which subsequently crystallised from light petroleum in plates, identical with a specimen of methyl veratrate, m. p. 58°.

(ii) When ayanin (0·5 g.) was heated under reflux for 8 hours with potassium hydroxide (2·8 g.) in water (25 c.c.) and alcohol (10 c.c.) in an atmosphere of hydrogen, yellow needles (0·3 g.) of the flavone potassium salt were obtained on cooling. After the salt had been collected, the filtrate was saturated with carbon dioxide and extracted with ether, and then strongly acidified and again extracted with ether. Both ethereal extracts yielded uncrystallisable oils of which the first, *i.e.*, the phenolic fraction, gave on vacuum-sublimation a small quantity of *iso*vanillic acid, identified by its mixed m. p.

3′-Benzyloxy-5 : 7-dihydroxy-3 : 4′-dimethoxyflavone (I).—Benzyl*iso*vanillic anhydride (4·3 g., 2·5 mols.) (Lovecy, Robinson, and Sugasawa, *J.*, 1930, 817), potassium benzyl*iso*vanillate (1·8 g., 1·6 mols.) and ω-methoxyphloracetophenone (0·75 g., 1 mol.) were thoroughly mixed and heated at 170—180°/30 mm. for 2 hours. The cooled product was powdered and dissolved in ethanol (36 c.c.) and water (3 c.c.) containing sodium hydroxide (3·7 g.), and the solution boiled for 10 minutes. The solvents were then removed under reduced pressure, and water (35 c.c.) and excess of acid added. The resulting emulsion was exhaustively extracted with ether, the ethereal solution washed with sodium hydrogen carbonate solution and evaporated, thus giving *3′-benzyloxy-5 : 7-dihydroxy-3 : 4′-dimethoxyflavone* (I) as a pale yellow solid (1·33 g., 89%) which crystallised from benzene in yellow plates, m. p. 211—212° (Found: C, 68·6; H, 5·2. $C_{24}H_{20}O_7$ requires C, 68·6; H, 4·8%).

3′-Benzyloxy-5-hydroxy-3 : 7 : 4′-trimethoxyflavone (II).—The 3′-benzylflavone (I) (0·35 g.) was methylated in dry acetone (18 c.c.) with methyl sulphate (0·08 c.c., 1·1 mols.) and anhydrous potassium carbonate (2 g.) under reflux for 6½ hours. The suspension was filtered, and the filtrate and acetone washings evaporated, leaving a brown solid (0·35 g.) which by recrystallisation from methanol gave *3′-benzylayanin* as orange needles, m. p. 138° (Found: C, 68·5; H, 5·2; OMe, 23·8. $C_{25}H_{22}O_7$ requires C, 69·1; H, 5·1; OMe, 21·4%).

5 : 3′-Dihydroxy-3 : 7 : 4′-trimethoxyflavone (*Ayanin*).—The 3′-benzylayanin (II) (0·15 g.) and a mixture of acetic acid (7 c.c.) and concentrated hydrochloric acid (3·7 c.c.) were heated on a steam-bath for 1½ hours. When the solution had been brought to neutrality with sodium hydroxide the *dihydroxytrimethoxyflavone* (III) (0·1 g., 89%) separated and by recrystallisation from methanol was obtained in orange-yellow plates, m. p. alone or mixed with the natural product, 173° (Found, in a specimen dried at 140° in a vacuum: C, 62·3; H, 4·3; OMe, 29·4%). Acetylation gave a diacetate, m. p. 115°, identical with diacetylayanin.

5 : 7 : 3′-Trihydroxy-3 : 4′-dimethoxyflavone (IV).—The benzyl ether (I) (0·1 g.) was heated in acetic acid (4·8 c.c.) and concentrated hydrochloric acid (2·6 c.c.) on a steam-bath for 1½ hours. Neutralisation of the solution and crystallisation of the precipitate (0·07 g., 90%) from methanol (charcoal) gave the *trihydroxydimethoxyflavone* (IV) as orange-brown plates, m. p., with slight previous softening, 232—233° (Found: C, 61·1; H, 4·4. $C_{17}H_{14}O_7$ requires C, 61·8; H, 4·3%).

3′-Benzyloxy-3 : 5 : 7 : 4′-tetramethoxyflavone (V).—The flavone (I) (0·3 g.) was heated under reflux for 24 hours with methyl iodide (5 c.c.) and acetone (50 c.c.) containing potassium carbonate (2 g.). After filtration, the solution was evaporated and the colourless product (0·32 g.) crystallised from ethanol, the *benzyloxytetramethoxyflavone* separating in plates, m. p. 143—144° (Found, in a specimen dried at 100° in a vacuum: C, 69·5; H, 5·5; OMe, 29·2. $C_{26}H_{24}O_7$ requires C, 69·6; H, 5·4; OMe, 27·7%).

3′-Hydroxy-3 : 5 : 7 : 4′-tetramethoxyflavone (VI).—Hydrolysis of the flavone (V) with a mixture of hydrochloric and acetic acids in the manner used for compounds (III) and (IV) gave *3′-hydroxy-3 : 5 : 7 : 4′-tetramethoxyflavone* which, when crystallised from methanol (charcoal), formed yellow elongated hexagonal plates, m. p. 220—222° (Found: C, 63·2; H, 5·3; OMe, 38·0. $C_{19}H_{18}O_7$ requires C, 63·7; H, 5·0; OMe, 34·6%).

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THE UNIVERSITY, NOTTINGHAM. [*Received, September 17th*, 1951.]

20. *The Chemistry of Extractives from Hardwoods. Part VI.* Constituents of Muninga, the Heartwood of* Pterocarpus Angolensis, *A.: 6 : 4′-Dihydroxy-5 : 7-dimethoxy*isoflavone (*Muningin*).

By F. E. King, T. J. King, and A. J. Warwick.

From alcoholic extracts of the heartwood of *Pterocarpus Angolensis* a new crystalline phenol, muningin, has been isolated. Through alkali fusion of the dimethyl ether, *p*-methoxyphenylacetic acid and 3 : 4 : 5-trimethoxyphenol are obtained, and the formation of *p*-ethoxyphenylacetic acid and of 4-ethoxy-3 : 5-dimethoxyphenol from the diethyl ether, with other confirmatory evidence, reveals the constitution of muningin to be 6 : 4′-dihydroxy-5 : 7-dimethoxy*iso*flavone.

Pterocarpus Angolensis is a tree of African origin which is the source of a hardwood known commercially as muninga. Only limited quantities have so far been exported but owing to its excellent general properties the timber has many potential applications in the building and furniture industries. As a wood of great natural durability it falls within the purview of our general investigations on the extractable components of resistant hardwoods and this paper gives an account of the isolation and identification of a phenolic constituent which we have termed muningin.

The genus *Pterocarpus* includes certain of the so-called insoluble redwoods, *e.g.*, red sandalwood (*P. santalinus*) and narrawood (*P. indicus*), which have already been extensively studied (see, for instance, McGookin, Robertson, and Whalley, *J.*, 1940, 787), but muninga and the very well-known commercial timbers Andaman padauk (*P. dalbergioides*) and Burma padauk (*P. macrocarpus*) have not hitherto been the subject of any chemical investigations. Unlike the padauks and the insoluble redwoods, however, muninga is not particularly conspicuous for its brilliant pigmentation, although the heartwood, which is golden- or light red-brown and streaked with lines of darker shade, is nevertheless remarkable for its handsome appearance.

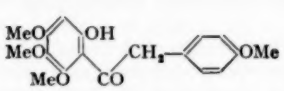
The powdered timber was exhaustively extracted in succession with light petroleum, ether, boiling chloroform, and boiling ethanol. The chloroform and the alcoholic extracts afforded a pure colourless compound, m. p. 285° (decomp.), termed muningin, the total yield being 0·55% of the heartwood. Subsequently, it was found that muningin could be satisfactorily isolated with boiling ethanol, without previous treatment with other solvents, the brown-red brittle resin on crystallisation from dioxan giving 0·7% of the pure compound.

Analyses of the intensively dried specimens of muningin together with a molecular-weight determination indicated the formula $C_{17}H_{14}O_6$. Normally, the compound and its principal derivatives contain water of crystallisation in amounts corresponding to a minimum unit cell of $2M,H_2O$, a value confirmed in the case of muningin by *X*-ray crystallographic measurements for which we are indebted to Dr. S. C. Wallwork. Muningin possesses two methoxyl groups, and the formation of dimethyl and diethyl ethers, and of a diacetate and dibenzoate, denoted the hydroxylic function of two further oxygen atoms. From the appearance of a pink colour on acidifying an alcoholic solution of muningin previously reduced by sodium amalgam, a reaction which did not occur with magnesium and hydrochloric acid, the presence of a flavone or *iso*flavone nucleus was inferred and the formation of an easily hydrolysed salt when muningin was added to concentrated hydrobromic acid gave further support to this view.

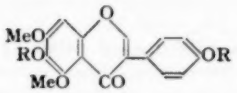
The isolation of picric acid from the vigorous reaction of concentrated nitric acid with muningin, and of a mixture of dinitroanisole and 4-methoxy-3-nitrobenzoic acid from the action of this reagent on dimethylmuningin, indicated the existence of a *p*-substituted hydroxyphenyl group. No other recognisable compound was obtained from these degradations, and the easy destruction of the larger part of the molecule was again observed when a permanganate oxidation of dimethylmuningin gave *p*-anisic acid as the sole isolable product.

* Part V, preceding paper.

Neither muningin nor dimethylmuningin showed carboxylic reactions, but on mild alkaline hydrolysis of the methyl ether formic acid was liberated, and the resulting crystalline product readily formed a semicarbazone. These results clearly established the *iso*flavone character of muningin and, when, as a result of a sodium hydroxide fusion of dimethylmuningin, *p*-methoxyphenylacetic acid and antiarol (3 : 4 : 5-trimethoxyphenol) were obtained, it was evident that the primary product of hydrolysis consisted of 2-hydroxy-4 : 5 : 6-trimethoxyphenyl 4-methoxybenzyl ketone (I), and that dimethylmuningin was therefore the 5 : 6 : 7 : 4′-tetramethoxy*iso*flavone (II; R = Me). A resynthesis of the latter from the ketone (I), ethyl formate, and sodium confirmed this interpretation.



(I)



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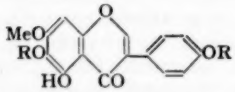

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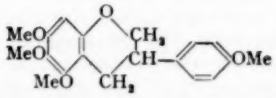

(IV)

Only the relative distribution of the hydroxy- and methoxy-groups at the 5-, 6-, 7-, and 4′-positions in muningin now remained to be elucidated. It is obvious from the oxidation results already quoted that one of the two hydroxyl groups occupies the 4′-position. Moreover, since muningin does not give a ferric reaction, the 5-substituent, which if phenolic would take part with the adjacent carbonyl in co-ordination complex formation, cannot be unmethylated. Hence the second hydroxyl group must be located at either the 6- or the 7-position. Its precise orientation became apparent from the degradation of muningin diethyl ether by alkali fusion, the formation of *p*-ethoxyphenylacetic acid and 4-ethoxy-3 : 5-dimethoxyphenol leading to 6 : 4′-dihydroxy-5 : 7-dimethoxy-*iso*flavone (II; R = H) as the structure of the natural product. For comparison we prepared 4-ethoxy-3 : 5-dimethoxyphenol by a method which is an improvement over that of Bogert and Ehrlich (*J. Amer. Chem. Soc.*, 1919, **41**, 798).

It was now possible to assign complete structures to certain demethylation and reduction products obtained earlier in the investigation. For example, the phenol obtained by heating muningin with a mixture of hydrobromic and acetic acids proved to have lost only one of the two methoxyl groups and, in view of the well-recognised effect of the carbonyl group on the stability of *o*-alkyloxy substituents, it may reasonably be concluded that this derivative is 5 : 6 : 4′-trihydroxy-7-methoxy*iso*flavone (III; R = H). When this compound was methylated under mild conditions the relatively unreactive 5-hydroxyl group was unaffected, the product being 5-hydroxy-6 : 7 : 4′-trimethoxy*iso*flavone (III; R = Me) identical with tectorigenin dimethyl ether (Shriner and Stephenson, *J. Amer. Chem. Soc.*, 1942, **64**, 2737).

Dimethylmuningin was reduced catalytically to a dihydro-derivative, evidently the corresponding *iso*flavanone, which afforded both an oxime and a 2 : 4-dinitrophenylhydrazone. The *iso*flavanone was further reduced by the Clemmensen reagent, but the boiling hydrochloric acid, unlike the mixture of hydrobromic and acetic acids, was without action on the 5-methoxyl group and the product is therefore regarded as 5 : 6 : 7 : 4′-tetramethoxy*iso*flavan (IV).

Experimental

Muningin.—The muninga used in these experiments was provided by the Forest Products Research Laboratory, and was made available through the generous assistance of the Director of the Laboratory and of Dr. W. P. K. Findlay.

(i) The powdered heartwood (1600 g.) which is sharply demarcated and easily distinguished from the pale sapwood, was first extracted with cold light petroleum (3 × 1 l.) and then with cold ether (12 × 5 l.), the respective extracts consisting of a yellow oil (10·8 g., 0·68%) and a dark red resin (89·4 g., 5·6%). This was followed by digestion with quantities of boiling chloroform (6 × 1 l.) until further treatment no longer extracted coloured material. Crystalline

solid separated during concentration of the combined chloroform solutions and, when the evaporated extract (48·7 g., 3·04%) was refluxed with methanol (100 c.c.), a microcrystalline solid (5·6 g.) was deposited after the mixture had been left at 0°. From this, muningin was obtained, by crystallisation from dioxan (charcoal), in colourless minute hexagonal plates, m. p. 285° (decomp.).

After chloroform extraction the timber was exhausted by boiling ethanol (3 × 5 l.). When the solvent had been evaporated the friable red-brown product (135 g.) remaining was refluxed with methanol (300 c.c.) and set aside at 0°. This afforded a crystalline solid (5 g.) which on recrystallisation (dioxan; charcoal) gave muningin (4 g., 0·25%) of m. p. 285° (decomp.); the total yield was 0·55%. Finally, the wood was digested with hot water, and the black amorphous extract (17 g., 1·05%) treated with 10% hydrochloric acid. Evaporation of the filtered acid solution and crystallisation of the residue from aqueous alcohol yielded potassium chloride (4·8 g.).

(ii) The powdered wood (6200 g.) was treated in batches (*ca.* 500 g.) for 6 hours with hot ethanol in a percolator. The combined extracts were evaporated to dryness and the residue (1150 g.) refluxed with methanol (1100 c.c.). After 12 hours at 0° the crude *muningin* (49 g.) was collected, and recrystallised from dioxan (charcoal), thus affording the pure compound (43·5 g., 0·7%), m. p. 285° (decomp.) (Found: C, 63·4, 63·1; H, 5·0, 4·8. $C_{17}H_{14}O_6,\frac{1}{2}H_2O$ requires C, 63·2; H, 4·6. Found, in a specimen dried in a vacuum at 150°: C, 64·8; H, 4·3. $C_{17}H_{14}O_6$ requires C, 65·0; H, 4·5%). Muningin is only very sparingly soluble in ether, chloroform, or ethanol, and slightly soluble in dioxan, ethanol, acetone, or acetic acid. It dissolves in aqueous sodium hydroxide to deep yellow solutions which become red on being warmed. It gives a deep red colour with sulphuric acid containing a trace of nitric acid. Prolonged treatment with sodium amalgam in alcohol is necessary before a pink colour is produced on acidification.

OO-*Diacetylmuningin*.—Muningin (0·1 g.) and fused sodium acetate (0·2 g.) were refluxed for 1 hour with acetic anhydride (2 c.c.), and the mixture was poured into water. Crystallisation of the precipitated product from acetic acid or ethanol gave *diacetylmuningin* (0·1 g.), as silky needles, m. p. 232—233° (Found, in a specimen dried in a vacuum at 110°: C, 61·7, 61·8; H, 4·7, 4·6. $C_{21}H_{18}O_8,\frac{1}{2}H_2O$ requires C, 61·9; H, 4·7%).

OO-*Dibenzoylmuningin*.—The oily product obtained by shaking a solution of muningin (0·1 g.) in 2N-sodium hydroxide (10 c.c.) with benzoyl chloride (0·2 g.) was thoroughly washed with water and dissolved in ethanol. *Dibenzoylmuningin* (0·12 g.) separated in long needles, m. p. 180° (Found: C, 70·2; H, 4·6. $C_{31}H_{22}O_8,\frac{1}{2}H_2O$ requires C, 70·1; H, 4·3. Found, in a specimen dried in a vacuum at 110°: C, 71·7; H, 4·6. $C_{31}H_{22}O_8$ requires C, 71·4; H, 4·2%).

OO-*Dimethylmuningin* (II; R = Me).—(i) A mixture of muningin (1 g.) and potassium carbonate (2 g.) was heated under reflux for 10 hours with acetone (100 c.c.) containing methyl iodide (5 c.c.). The filtered solution was then evaporated, and the residue washed with water and crystallised from methanol. *Dimethylmuningin* (0·84 g., 81%) separated in prisms, m. p. 176° [Found: C, 65·2; H, 5·4%; unit cell, 678. $C_{19}H_{18}O_6,\frac{1}{2}H_2O$ requires C, 64·8; H, 5·4%; unit cell, 693. Found, in a specimen dried in a vacuum at 50°: C, 66·3; H, 5·2; OMe, 37·8%; *M* (Rast), 324. $C_{15}H_{10}O_2(OMe)_4$ requires C, 66·6; H, 5·3; OMe, 36·2%; *M*, 342].

(ii) A solution of muningin (1 g.) in 2N-sodium hydroxide was shaken for 2 hours at room temperature with methyl sulphate (2 c.c.). Crystallisation of the precipitate from methanol gave the dimethyl ether (0·7 g., 68%), m. p. and mixed m. p. 175—176°.

OO-*Diethylmuningin* (II; R = Et).—Prepared by the ethyl iodide–potassium carbonate method, *diethylmuningin* (yield 67%) crystallised from ethanol in needles, m. p. 156° (Found: C, 67·9; H, 6·1. $C_{21}H_{22}O_6$ requires C, 68·1; H, 5·9%).

Nitric Acid Oxidation of Muningin and of Dimethylmuningin.—Addition of concentrated nitric acid (5 c.c.) to muningin (0·5 g.) caused a vigorous reaction. The mixture was then warmed on a steam-bath for 15 minutes and poured into water (20 c.c.). Ether-extraction isolated a product which was completely soluble in aqueous sodium hydrogen carbonate and on crystallisation from alcohol had m. p. 120° alone or mixed with a specimen of picric acid.

Dimethylmuningin (0·4 g.) was warmed with concentrated nitric acid (5 c.c.) on a steam-bath for 30 minutes and the liquid then poured into water (20 c.c.). The resulting solution was extracted with ether (3 × 10 c.c.), and the combined extracts were washed with aqueous sodium hydrogen carbonate. Evaporation of the dried ethereal layer gave a pale yellow oil which crystallised from light petroleum in colourless needles, m. p. 84—86°. After recrystallisation the product (0·03 g.) had m. p. 87°, undepressed by 2 : 4-dinitroanisole prepared by the method of Meldola, Woolcott, and Wray (*J.*, 1896, **69**, 1330).

The bicarbonate solution was acidified, and then yielded to ether a pale yellow solid which crystallised from water in colourless needles (0·12 g.), m. p. 185—187° undepressed by an authentic specimen of 4-methoxy-3-nitrobenzoic acid.

Oxidation of Dimethylmuningin with Potassium Permanganate.—No reaction occurred when dimethylmuningin (0·5 g.) dissolved in cold acetone (50 c.c.) was treated with powdered potassium permanganate, but warming the mixture under reflux yielded a copious precipitate and the addition of reagent was continued until a slight excess remained. The precipitate was collected and digested with boiling water (2 × 10 c.c.), and the resulting solution filtered and acidified The light brown solid thus obtained crystallised from water in colourless long needles (0·13 g.), m. p. and mixed m. p. with *p*-anisic acid, 187°. The acetone filtrate was also examined but contained no organic compound.

Alkaline Hydrolysis of Dimethylmuningin.—Dimethylmuningin (1 g.) was heated under reflux for 30 minutes with ethanol (50 c.c.) containing potassium hydroxide (2 g.). When the solution was made slightly acid with dilute sulphuric acid and the alcohol distilled, an oil separated which was collected in ether. Further concentration of the aqueous solution gave an acid distillate which rapidly precipitated silver from Tollens's reagent. Distillation of the dried ethereal extract afforded a yellow viscous syrup (0·82 g.), b. p. 190—200° (bath-temp.)/1 mm. which crystallised from light petroleum in colourless plates, m. p. 73° (Found: C, 64·6; H, 5·7. $C_{18}H_{20}O_6$ requires C, 64·9; H, 6·0%). The product, *6-hydroxy-2 : 3 : 4-trimethoxyphenyl 4-methoxybenzyl ketone* (I), gave a green ferric reaction. It formed a *semicarbazone* which separated from aqueous ethanol in needles, m. p. 181° (Found: N, 10·9, 11·0. $C_{19}H_{23}O_6N_3$ requires N, 10·8%).

Dimethylmuningin was resynthesised when the ketone (I) (1·7 g.) was shaken for 6 hours at 0° with anhydrous ethyl formate (30 c.c.) containing powdered sodium (0·5 g.) and then left overnight in a refrigerator. The mixture was poured on ice and vigorously stirred to evaporate the excess of ethyl formate, the product being isolated by ether-extraction. Crystallised from methanol, the *iso*flavone (0·35 g., 20%) had m. p. and mixed m. p. 175°.

Degradation of Dimethylmuningin by Alkali Fusion.—A mixture of dimethylmuningin (2 g.), sodium hydroxide (5 g.) and water (2 c.c.) in a copper tube fitted with an air-condenser was heated for 45 minutes at 220° (oil-bath). When cold, the product was dissolved in water which was acidified with hydrochloric acid, and the solution extracted with ether (4 × 50 c.c.). The ethereal extract, when washed with sodium hydrogen carbonate solution and dried, gave on evaporation a reddish product crystallising from benzene in long colourless needles (0·31 g., 29%), m. p. 147° alone or mixed with a specimen of antiarol prepared by the method of Chapman, Perkin, and Robinson (*J.*, 1927, 3028) (Found: C, 58·7; H, 6·4. Calc. for $C_9H_{12}O_4$: C, 58·7; H, 6·5%). It gave pale yellow-green colour with ferric chloride, and its acetate had the recorded m. p. of 74°.

The bicarbonate washings of the ethereal extract were acidified and shaken with ether. Evaporation of the dried ethereal solution gave an oil which crystallised from light petroleum in colourless plates (0·45 g., 47%) consisting of *p*-methoxyphenylacetic acid, m. p. and mixed m. p. 85—86°.

Alkali Degradation of Diethylmuningin.—Diethylmuningin (1 g.), sodium hydroxide (5 g.) and water (2 c.c.), treated as described for the dimethyl ether, gave, from the ethereal extract, colourless needles, m. p. 118°, consisting of 4-ethoxy-3 : 5-dimethoxyphenol (Found: C, 60·9; H, 7·1. Calc. for $C_{10}H_{14}O_4$: C, 60·6; H, 7·1%). Acidification of the bicarbonate washings and ether extraction gave *p*-ethoxyphenylacetic acid (Warmer, *Annalen*, 1902, **322**, 149), m. p. and mixed m. p. 84°.

4-Ethoxy-3 : 5-dimethoxyphenol.—The preparation of this phenol by Bogert and Ehrlich's method (*loc. cit.*) is unsatisfactory at the final stage, *viz.*, diazotisation, etc., of the 4-ethoxy-3 : 5-dimethoxyaniline. It is more conveniently obtained by the following adaptation of the Chapman, Perkin, and Robinson synthesis (*loc. cit.*). 2 : 6-Dimethoxyquinol (1 g.) was dissolved in 10% aqueous sodium hydroxide (25 c.c.) containing a trace of sodium dithionite, and the solution shaken with ethyl sulphate (1·4 c.c., 2 mols.) for 1 hour. After extraction with ether, the solution was acidified and the brown oil collected with ether and crystallised from light petroleum. Further crystallisation from water, which was attended by considerable loss, gave 4-ethoxy-3 : 5-dimethoxyphenol in needles (0·22 g., 19%), m. p. 118°, alone or mixed with a specimen obtained from diethylmuningin.

*5 : 6 : 4′-Trihydroxy-7-methoxy*isoflavone (III; R = H).—When muningin (1 g.) was dissolved in a mixture of hydrobromic acid (15 c.c.; *d* 1·5) and acetic acid (5 c.c.) a yellow precipitate—presumably the hydrobromide—immediately separated. The mixture was

heated, whereupon the precipitate dissolved to be followed shortly afterwards by the separation of the *trihydroxy*-compound (0·88 g.) which crystallised from acetic acid or ethanol in clusters of prisms, m. p. 258° (decomp.) (Found: C, 62·1, 61·8; H, 4·4, 4·4; OMe, 9·2. $C_{15}H_9O_5 \cdot OMe, \frac{1}{2}H_2O$ requires C, 62·1; H, 4·2; OMe, 9·2%). The *iso*flavone gave a deep green colour with ferric chloride.

5-Hydroxy-6 : 7 : 4′-trimethoxyisoflavone (III; R = Me).—The foregoing compound (0·1 g.), potassium carbonate (1 g.), methyl iodide (2 c.c.), and acetone (50 c.c.) were refluxed for 10 hours and the product was worked up in the usual way. When crystallised from ethanol 5-hydroxy-6 : 7 : 4′-trimethoxy*iso*flavone (0·09 g.) formed needles, m. p. 186° (lit., 188°) (Found: C, 64·6; H, 5·2. Calc. for $C_{18}H_{16}O_6, \frac{1}{2}H_2O$: C, 64·2; H, 5·0%). It is very sparingly soluble in methanol, insoluble in aqueous sodium hydroxide, and gives a deep green ferric reaction.

When methylation was effected with methyl sulphate and potassium carbonate in boiling acetone for 30 hours, the product (yield 56%) was muningin dimethyl ether, m. p. and mixed m. p. 176°.

Dihydromuningin Dimethyl Ether (*5 : 6 : 7 : 4′-Tetramethoxyisoflavanone*).—Dimethylmuningin (0·8 g.) in ethanol (40 c.c.), in a catalytic reduction over palladised charcoal (0·1 g.), absorbed 55 c.c. of hydrogen (theory, 56·2 c.c.) in 35 minutes. When the solution was filtered and concentrated to 5 c.c., the iso*flavanone* (0·62 g., 77%) crystallised in rosettes of needles, m. p. 147—148° (Found: C, 65·9; H, 5·6. $C_{19}H_{20}O_6$ requires C, 66·2; H, 5·8%). In warm ethanolic 2 : 4-dinitrophenylhydrazine sulphate, this gave deep red rods of the *2 : 4-dinitrophenylhydrazone*, m. p. (after crystallisation from ethanol) 165—166° (Found: C, 56·3; H, 4·7; N, 9·9. $C_{25}H_{24}O_9N_4, \frac{1}{2}H_2O$ requires C, 56·3; H, 4·7; N, 10·5. Found, in a specimen dried in a vacuum at 100°: C, 57·6; H, 4·7. $C_{25}H_{24}O_9N_4$ requires C, 57·2; H, 4·6%). The *oxime* crystallised from ethanol in rhombic plates, m. p. 193° (Found: C, 63·2, 63·4; H, 6·2, 6·2; N, 4·0. $C_{19}H_{21}O_6N$ requires C, 63·4; H, 5·9; N, 3·9%).

5 : 6 : 7 : 4′-Tetramethoxyisoflavan (IV).—Amalgamated zinc dust (5 g.) was covered with acetic acid (15 c.c.) and dihydromuningin dimethyl ether (0·3 g.) added. The mixture was gently refluxed for 8 hours with the occasional addition of concentrated hydrochloric acid (total, 10 c.c.). The product was extracted from the cooled solution by ether which when washed with water, dried, and evaporated gave the iso*flavan* (0·22 g.), crystallising from methanol as a microcrystalline powder, m. p. 95—96° (Found: C, 69·1; H, 6·5. $C_{19}H_{22}O_5$ requires C, 69·0; H, 6·7%). The *iso*flavan was insoluble in aqueous sodium hydroxide and gave a negative ferric reaction.

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21. *The Isomerisation of* p-*Xylene.*

By G. BADDELEY, G. HOLT, and D. VOSS.

The presence of a third component such as hydrogen halide is shown to be an essential feature of the interaction of an aromatic hydrocarbon and an aluminium halide at room temperature. Mixtures of aluminium bromide and *m*- or *p*-xylene are shown to absorb hydrogen bromide until the relationship $HBr : AlBr_3 = 1 : 1$ obtains. Ternary complexes of the type $(ArH)_x(HBr,AlBr_3)_y$ have been obtained for the first time, and an almost colourless crystalline complex which provides analytical data compatible with the formula $(m\text{-Xylene})_3(HBr,AlBr_3)_2$ has been obtained from *m*-xylene and also from *p*-xylene.

ALTHOUGH the rate of isomerisation of the xylenes in the presence of aluminium chloride is reported to be unaffected by the presence of hydrogen chloride (Norris and Vaala, *J. Amer. Chem. Soc.*, 1939, **61**, 2131), one of us has demonstrated that hydrogen chloride is necessary in the isomerisation of phenols, aromatic ketones, and hydroxy-aromatic ketones (*J.*, 1950, 994). This contradiction led us to reinvestigate the interaction of

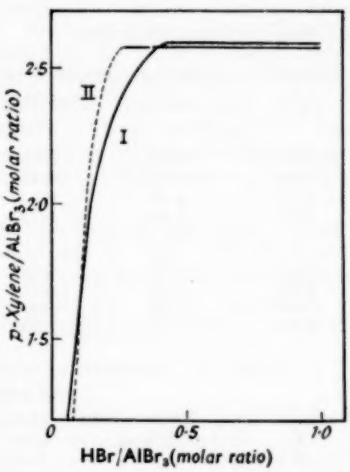
aluminium bromide with *p*-xylene and other aromatic hydrocarbons; the bromide was selected because it is readily soluble in the hydrocarbons.

Evaporation causes precipitation of the bromide from a solution of aluminium bromide in toluene (Norris and Rubinstein, *J. Amer. Chem. Soc.*, 1939, **61**, 1163), and Korshak, Lebedev, and Fedoseev (*Chem. Abs.*, 1948, **42**, 1217) have deduced, from the strict additivity of the molar refractivities of solutions of the bromide in both benzene and toluene, that no definite complexes are formed. Van Dyke (*J. Amer. Chem. Soc.*, 1950, **72**, 3619), using freezing-point data, has shown that aluminium bromide exists as Al_2Br_6

TABLE 1. *Products of interaction of hydrogen bromide, aluminium bromide, and aromatic hydrocarbons.*

Hydrocarbon	Complex	Description	Effect of diminished pressure: Press. (mm.)	Residue
Benzene	$(C_6H_6)_6,Al_2Br_6,HBr$	Oil	12	Al_2Br_6
Toluene	$(C_7H_8)_6,Al_2Br_6,HBr$	Oil	12	$C_7H_8Al_2Br_6$
			0·002	mainly Al_2Br_6
Mesitylene	$(C_9H_{12})_2,Al_2Br_6,HBr$	Solid	0·002	C_9H_{12},Al_2Br_6
s-Triethylbenzene	$(C_{12}H_{18})_2,Al_2Br_6,HBr$	Solid	0·002	$C_{12}H_{18},Al_2Br_6$

in solution in benzene; he claims that the more concentrated solutions provide a solid phase of $Al_2Br_6,2C_6H_6$ and suggests that this is the form in which the bromide is present in solution (cf. Plotnikov and Gratsianskiv, *Chem. Abs.*, 1939, **33**, 243). Gustavson (*Ber.*, 1878, **11**, 2151; 1879, **12**, 593) showed that aluminium chloride combines with benzene and toluene in the presence of hydrogen chloride to provide orange-coloured oils which regenerate the hydrocarbon on addition of water. Norris and Ingraham (*J. Amer. Chem. Soc.*, 1940, **62**, 1298) obtained complexes of the type $(ArH)_n,Al_2Br_6,HBr$ by bubbling hydrogen bromide into solutions of aluminium bromide in aromatic hydrocarbon; several complexes are listed in Table 1 and similar ones have been obtained with aluminium and hydrogen chlorides. The products obtained by reducing the pressure are given in the last column; they show that the stability of these complexes is a minimum for benzene and increases progressively as alkyl groups are introduced.

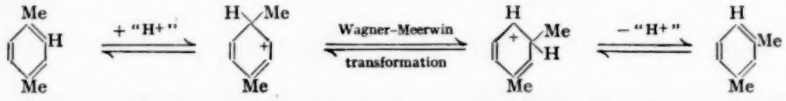


The literature does not contain the relevant data for the xylenes; Campbell and Hetherington (*Nature*, 1945, **156**, 389) have shown, however, that *m*-xylene forms a more stable complex with aluminium chloride than does *p*-xylene, and Campbell and Eley (*ibid.*, 1944, **154**, 85) have recorded the following heats of complex formation of aluminium chloride with benzene homologues: xylene, 22; mesitylene, 8; toluene, 0 kcal. Unfortunately, these authors do not refer to the presence of hydrogen chloride.

Our investigation of the effect of hydrogen bromide on the rate of isomerisation of *p*-xylene required homogeneous reaction mixtures and therefore entailed a preliminary study of ternary systems of *p*-xylene, aluminium bromide, and hydrogen bromide. Curve I (see figure) shows the composition of the dark oil deposited when various amounts of hydrogen bromide were dissolved in a solution of aluminium bromide in *p*-xylene (5 mols., based on the formula $AlBr_3$ for aluminium bromide) at 17·0°. The system was saturated with hydrogen bromide when the ratio $HBr : AlBr_3 = 1 : 1$ was reached and the complex now contained *ca.* 2·7 mols. of *p*-xylene; this was only little more than the amount of *p*-xylene (2·5 mols.) present in the complex when much less hydrogen bromide (*e.g.*, 0·30 mol.) was present. Curve II was obtained with *p*-xylene (2·56 mols.); the system was completely homogeneous when the amount of hydrogen bromide exceeded

0·25 mol. and, once again, could absorb up to 1·00 mol. of hydrogen bromide. Systems represented by the broken part of the curve contain undissolved aluminium bromide.

Isomerisation of p-*xylene.* This hydrocarbon remained unchanged after contact with excess of aluminium bromide at room temperature for a day. After a day at room temperature, two homogeneous reaction mixtures containing aluminium bromide, *p*-xylene (2·56 mols.), and hydrogen bromide (0·33 and 0·52 mol., respectively) provided hydrocarbon which was analysed by infra-red and ultra-violet spectroscopy; the data are assembled in Table 2, which also presents the results of four experiments in which a greater variation in concentration of hydrogen bromide was made and in which the reaction mixtures were placed in a thermostat at 25·0°. *All these data show that the rate of isomerisation of* p-*xylene increases with the concentration of hydrogen bromide.* The absence of any considerable disproportionation shows that isomerisation is not due to demethylation and subsequent remethylation and supports the mechanism proposed by one of us (*loc. cit.*):



Ternary complexes of aromatic hydrocarbon, aluminium bromide, and hydrogen bromide in which the ratio HBr : $AlBr_3$ = 1 : 1 obtains are novel, only complexes in which the ratio HBr : Al_2Br_6 = 1 : 1 obtains having been described previously, and we have now measured the solubility of hydrogen bromide in mixtures of aluminium bromide with benzene, toluene, and *m*- and *p*-xylene, severally. The data are assembled in Table 3 and are in agreement with a decrease in basicity of these hydrocarbons in the order xylene > toluene > benzene, basicity being defined in the Lewis sense as ability to donate a pair of electrons.

TABLE 2. *Extent of isomerisation in ternary complexes of* p-*xylene, aluminium bromide, and hydrogen bromide after* 24 *hours.*

At room temperature.

p-Xylene (mols.)	$AlBr_3$ (mol.)	HBr (mol.)	Products, % PhMe	*o*-Xylene	*m*-Xylene	*p*-Xylene
2·56	1·00	0·33	<1·0	0·8	38	61
2·56	1·00	0·52	<1·0	1·6	53	45
			At 25·0°.			
2·50	1·00	0·43	1·5	1·5	45	46
2·50	1·00	0·64	<1·0	2·5	58	37
2·50	1·00	1·00	<1·0	3·0	66	28
2·50	1·00	1·00	1·0	2·0	67	27

TABLE 3. *Solubility of hydrogen bromide in mixtures of aluminium bromide and aromatic hydrocarbon at* 17·0°.

Expt.	Hydrocarbon	(mole)	$AlBr_3$ (mole)	HBr (mole)	HBr/$AlBr_3$
1	*p*-Xylene	0·071	0·028	0·029	1·0
2	,,	0·080	0·032	0·031	1·0
3	,,	0·096	0·038	0·038	1·0
4	*m*-Xylene	0·086	0·048	0·047	1·0
5	,,	0·150	0·034	0·033	1·0
6	Toluene	0·084	0·027	0·021	0·8
7	Benzene	0·140	0·039	0·022	0·6

A crystalline complex was obtained from *m*-xylene. In Expt. 4 the initial dark oil soon changed into a mass of small, pale brown, tetrahedral crystals. A larger proportion of *m*-xylene was used in Expt. 5 and large (*ca.* 1 cm.) tetrahedral crystals (m. p. 52—54°) were slowly deposited, having a composition compatible with the formula $(m\text{-Xylene})_3(AlBr_3,HBr)_2$. The loss of colour accompanying crystallisation is very remarkable: it is obviously related to Norris and Ingraham's observation (*loc. cit.*) that the complex $(1:3:5\text{-}Et_3C_6H_3)_2,Al_2Br_6,HBr$ is a red oil which changes into yellow crystals, and should be borne in mind when attempting to explain the colour of liquid complexes of aromatic

hydrocarbons with aluminium and hydrogen halide and of solutions of toluene and higher benzene homologues in anhydrous hydrogen fluoride (Klatt, *Z. anorg. Chem.*, 1937, **234**, 189; see Dewar, " Electronic Theory of Organic Chemistry," Oxford, 1949, p. 169).

The homogeneous mixtures obtained in Expts. 1, 2, and 3 had separated into three phases after 4 days at 17° : a top layer of hydrocarbon, a middle layer of dark oil, and a bottom layer of large and almost colourless tetrahedral crystals (m. p. 52—54°) which did

TABLE 4.

	Crystalline complex :		$(C_8H_{10})_3(AlBr_3,HBr)_2$	Liquid complex :		$(C_8H_{10})_3AlBr_3,HBr$
	Expt. 1	Expt. 2	requires	Expt. 1	Expt. 2	requires
Hydrocarbon, %	30·0	30·9	31·4	47·0	47·2	47·7
Br, %	63·9	63·6	63·1	47·3	47·8	48·0
Al, %	5·6	5·4	5·3	4·1	4·1	4·05

Composition of the hydrocarbons obtained from the three phases.

	Crystalline complex	Liquid complex	Supernatant hydrocarbon
o-Xylene, %	0·5	5·3	3·6
m-Xylene, %	96·0 *	66·5	39·0
p-Xylene, %	3·5	26·0	58·0
Toluene, %	<1·0	2·5	<0·5
Total	100·0	100·3	100·6

* The impurities in the *m*-xylene are probably due to slight contamination of the crystalline complex by the liquid complex.

not depress the melting point of those obtained from *m*-xylene. The three phases were separated and analysed; the results are assembled in Table 4, and are compatible with the formulæ $(m\text{-Xylene})_3(AlBr_3,HBr)_2$ and $(Xylene)_3(AlBr_3,HBr)$ for the crystalline complex and the liquid complex, respectively.

All the ternary complexes listed in Table 3 were stable only in an atmosphere of hydrogen bromide and lost part of this component when subjected to a stream of dry nitrogen; *e.g.*, the crystalline complex $(m\text{-Xylene})_3(AlBr_3,HBr)_2$ and the liquid complex $(p\text{-Xylene})_{2.5}(AlBr_3,HBr)$ readily lost half their hydrogen bromide and provided homogeneous dark oils of composition $(ArH)_xAl_2Br_6,HBr$; these correspond to those obtained previously by other investigators. The complex from benzene readily lost most of its hydrogen bromide (75%) and separated into benzene and a solid phase (see Van Dyke, *loc. cit.*).

Ethylbenzene, propylbenzene, naphthalene, and tetralin suffered only little change in the presence of aluminium bromide at room temperature; in each instance reaction occurred when hydrogen bromide was also present. Ethylbenzene and propylbenzene suffered disproportionation into benzene and higher homologues, and ββ-dinaphthyl and *s*-octahydroanthracene were identified among the products obtained from naphthalene and tetralin, respectively.

EXPERIMENTAL

Freshly redistilled aluminium bromide (4·70 g.) was dissolved in *p*-xylene (10·60 g.; m. p. 11·3°) in a graduated cylinder fitted with a ground-joint stopper carrying inlet and outlet tubes fitted with taps. The inlet tube reached to the *bottom* of the cylinder. Hydrogen bromide was gradually bubbled into the mixture from a drying tower packed with phosphoric oxide. The mixture was shaken in a thermostat at 17·0° and the weight of hydrogen bromide absorbed and the volume of ternary complex and of supernatant hydrocarbon were determined after selected intervals of time. The results were as follows :

HBr (g.)	$HBr/AlBr_3$	Ternary complex (c.c.)	Supernatant hydrocarbon (c.c.)	$C_6H_4Me_2$ in complex/$AlBr_3$
0·079	0·055	4·6	9·7	1·2
0·137	0·096	5·6	8·7	1·7
0·299	0·209	7·0	7·3	2·3
0·467	0·327	7·5	6·8	2·5
0·627	0·439	7·6	6·7	2·6
0·910	0·636	7·7	6·7	2·6
1·200	0·839	7·8	6·7	2·6
1·430	1·00	7·8	6·7	2·6

The above experiment was repeated with aluminium bromide (8·40 g.) and *p*-xylene (8·60 g.). The bromide was not wholly in solution until the supernatant hydrocarbon had disappeared :

HBr (g.)	$HBr/AlBr_3$	Ternary complex (c.c.)	Supernatant hydrocarbon (c.c.)	$C_6H_4Me_2$ in complex/$AlBr_3$
0·128	0·051	6·6	6·6	0·9
0·239	0·094	8·6	4·6	1·4
0·305	0·118	9·9	3·3	1·7
0·468	0·184	12·5	0·7	2·4
0·687	0·271	13·2	0·0	2·58
2·550	1·00	13·4	0·0	2·58

After 4 days at 18°, large tetrahedral crystals and supernatant hydrocarbon (1·5 c.c.) had separated. These are described more fully below.

After 4 days at 18°, a homogeneous mixture of *p*-xylene (10·2 g.), aluminium bromide (10·2 g.), and hydrogen bromide (3·2 g.) had separated into three phases : supernatant hydrocarbon, dark brown oil, and large, pale brown, tetrahedral crystals. The reaction vessel was fitted with a separating funnel into which, after 7 days, the liquid phases were decanted by inverting the apparatus. The crystals (m. p. 52—54°) did not depress the m. p. of the ternary crystalline complex obtained from *m*-xylene, aluminium bromide, and hydrogen bromide. The three phases were separated and weighed amounts of the solid and of the liquid complex were decomposed with ice and water without loss of hydrogen bromide. In each instance, the hydrocarbon was isolated and its composition determined by infra-red and ultra-violet spectroscopy. The bromide and the aluminium content were determined respectively by titration with 0·2N-alkali and by precipitation with 8-hydroxyquinoline. The results are presented in Table 4.

Effect of Hydrogen Bromide on the Rate of Isomerisation of p-*Xylene.*—The hydrocarbon (9·5 g. ; m. p. 11·3°) was added to aluminium bromide (11·6 g.) in a flask fitted with a ground-glass stopper. A part of the bromide dissolved and the mixture provided *p*-xylene (8·7 g. ; m. p. 9·5°) after a day at 17°. The same result was obtained when potassium bromide was also present.

p-Xylene (17·4 g.) was added to aluminium bromide (17·1 g.), and hydrogen bromide (1·70 g.) was added until the mixture was homogeneous. Half this mixture was transferred to a second vessel and hydrogen bromide (0·50 g.) was added. Both mixtures were decomposed with water after 24 hours at 17°. Four mixtures were decomposed after 24 hours at 25·0° ; they had contained the following weights of *p*-xylene, aluminium bromide, and hydrogen bromide, respectively : (*a*) 7·95, 8·01, and 1·05 g. ; (*b*) 10·17, 10·15, and 2·02 g. ; (*c*) 6·20, 6·13, and 1·86 g. ; (*d*) 5·30, 5·08, and 1·54 g. The composition of the recovered hydrocarbons was determined (see Table 2).

Solubility of Hydrogen Bromide in Mixtures of Aluminium Bromide and Aromatic Hydrocarbon.—Benzene. Benzene (10·9 g.), which had been shaken several times with concentrated sulphuric acid and finally distilled from sodium, was added to freshly distilled aluminium bromide (10·5 g.) at 17·0°. Much of the bromide did not dissolve until hydrogen bromide was added ; the mixture absorbed 1·85 g. and provided a dark brown oil. A stream of dry (P_2O_5) nitrogen (50 c.c./min.) was passed in turn through a bubbler containing benzene, the mixture, a closely packed plug of glass wool, and finally an absorption vessel containing water which was neutralised periodically with 0·1N-alkali. In 24 hours, alkali (170 c.c. ; equivalent to 1·38 g. of hydrogen bromide) was required. The initially homogeneous mixture had partly decomposed into a surface layer of benzene and a crystalline bottom layer (cf. Van Dyke, *loc. cit.*).

Toluene. This hydrocarbon (7·7 g.) and aluminium bromide (7·3 g.) absorbed hydrogen bromide (1·75 g.) and provided a homogeneous brown oil from which, after 24 hours at 17·0°, the toluene was recovered quantitatively.

p-*Xylene.* *p*-Xylene (7·5 g. ; m. p. 11·3°) and aluminium bromide (7·5 g.) provided a mixture which absorbed hydrogen bromide (2·3 g.). A stream of dry nitrogen (50 c.c./min.), in 24 hours, removed hydrogen bromide (1·05 g.) which neutralised 130 c.c. of 0·1N-alkali. The mixture was decomposed with dilute hydrochloric acid and provided hydrocarbon (6·6 g. ; m. p. −6°) which apparently contained 85% of *p*-xylene.

m-*Xylene.* This isomer (9·1 g.) was added to aluminium bromide (12·9 g.) ; most of the bromide dissolved and the rest soon dissolved on addition of dry hydrogen bromide. The mixture absorbed hydrogen bromide (3·85 g.) and pale brown crystals were deposited until only

a trace of dark brown oil remained. A stream of dry nitrogen (50 c.c./min.) swept out hydrogen bromide (2·00 g.) in 2 days and a dark oil remained. The experiment was repeated with *m*-xylene (16·2 g.) and aluminium bromide (9·0 g.); the mixture absorbed hydrogen bromide (2·7 g.) and formed a dark brown oil with a supernatant layer of colourless hydrocarbon. This layer increased in volume during 12 hours at 17·0° and large, pale brown, tetrahedral crystals (m. p. 52—54°) were deposited from the dark brown oil.

Effect of Hydrogen Bromide on the Interaction of Other Hydrocarbons with Aluminium Bromide.—*Ethylbenzene.* This hydrocarbon (8·5 g.) was added to aluminium bromide (11·4 g.), and the mixture kept at 17° for 2 days. Ethylbenzene (b. p. 134—134·5°; 6·8 g.) was recovered. Ethylbenzene (10·9 g.), aluminium bromide (10·1 g.), and hydrogen bromide (0·9 g.) provided a homogeneous dark oil which was not saturated with hydrogen bromide and, after 2 days at 17°, provided the following fractions: (i) benzene (b. p. 80—85°; 0·5 g.), (ii) ethylbenzene (b. p. 134—136°; 1·0 g.), (iii) *m*- and *p*-diethylbenzene (b. p. 170—180°; 6·5 g.), (iv) higher homologues (b. p. 205—210°; 1·8 g.); (v) residue (0·5 g.) (see Heise and Töhl, *Annalen*, 1872, **270**, 158).

Propylbenzene. (i) This (b. p. 160°; 6·0 g.) was recovered from a mixture of propylbenzene (7·1 g.) and aluminium bromide (9·4 g.) which had been kept at 17° for 24 hours. (ii) Propylbenzene (9·6 g.), aluminium bromide (9·9 g.), and hydrogen bromide (0·8 g.) formed a homogeneous dark red oil which provided fractions containing benzene (0·8 g.), propylbenzene (0·7 g.), dipropylbenzene (4·8 g.), and higher-boiling products (2·2 g.) (see Heise and Töhl, *loc. cit.*).

Tetralin. This hydrocarbon (15·8 g.) was recovered from a mixture of tetralin (18·3 g.) and aluminium bromide (7·6 g.) which had been kept at 17° for 12 hours. Tetralin (10·8 g.), aluminium bromide (11·1 g.), and hydrogen bromide (2·1 g.) formed a homogeneous deep red oil which, after 12 hours at 17°, provided the following hydrocarbon fractions upon distillation under 15 mm.: (i) tetralin (b. p. 85—88°; 1·8 g.); (ii) mainly octahydroanthracene (m. p. and mixed m. p. 72—73°; b. p. 160—170°; 2·9 g.), (iii) 190—200° (2·8 g.); (iv) residue (2·4 g.) (see Schroeter, *Ber.*, 1924, **57**, 1990).

Naphthalene. Naphthalene (4·8 g.) and aluminium bromide (6·8 g.) in chlorobenzene (30 c.c.) provided a blood-red solution from which naphthalene (4·2 g.) was recovered after 12 hours at 17°. Naphthalene (4·5 g.), aluminium bromide (7·3 g.), and hydrogen bromide (0·5 g.) in chlorobenzene (30 c.c.) provided a very dark solution from which no naphthalene and a little ββ-dinaphthyl were obtained after 12 hours at 17°.

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22. *The Reaction of Mercuric and Mercurous Nitrates with Formic Acid.*

By A. R. TOPHAM and A. G. WHITE.

The kinetics of the reduction of mercuric and mercurous nitrates by formic acid in aqueous solutions have been studied under a wide range of conditions. The effect on the rates of reaction of temperature, hydrogen-ion concentration, and formic acid concentration have been studied at a constant ionic strength. The results obtained indicate that both hydrolysed and unhydrolysed mercury ions take part in the reaction and that only the formate ion, and not undissociated formic acid, is capable of causing the reduction.

THE reaction of formic acid with the cobaltic ion has recently been studied by Bawn and White (*J.*, 1951, 339), who conclude that the most probable rate-determining steps are electron-transfer reactions between the cobaltic ion and formic acid or formate ion. It therefore seemed of interest to study analogous reactions of the formate ion in the hope of obtaining some information on the factors controlling such electron-transfer processes. The reactions of formic acid with mercury and silver salts seemed to be suitable, and the

present paper presents a detailed examination of the kinetics of the reactions of mercuric and mercurous nitrates with formic acid in aqueous solution.

The reaction of formic acid with mercuric chloride has been investigated by Dhar (*J.*, 1917, **111**, 707), but his results are of little value for the present purposes. Control of experimental conditions was not sufficiently rigorous and the use of the largely covalent mercuric chloride introduced complications. Consequently, we have re-examined the reaction. In an attempt to ensure the presence of mercuric and mercurous ions, the nitrates were used in an aqueous medium of nitric acid–potassium nitrate at constant ionic strength. In the course of the work, it soon became clear that the mercurous ion formed by the reaction of mercuric ion was also reduced by formic acid as shown by the precipitation of metallic mercury in the final stages of the reaction; it was this which prompted the extension of the investigation to the mercurous salt. Fortunately, the reaction rates of the mercuric and the mercurous ion proved to differ sufficiently for the two reactions to be studied independently.

The data obtained for the two reactions are presented in the following section and then discussed jointly, as a similar interpretation is possible in both cases.

Experimental

Materials.—"AnalaR" Reagents were used throughout, and no further purification was attempted.

Analytical Method.—Determination of mercuric ion in aqueous nitric acid solution in the presence of mercurous ion was carried out by a method based on that described by Warshowsky and Elving (*Anal. Chem.*, 1947, **19**, 112), *i.e.*, oxidation of mercurous to mercuric ion by reaction with iodine and titration of excess of iodine with thiosulphate. The mercuric-ion concentration could be calculated from the known initial mercuric-ion concentration and the concentration of mercurous ion found by titration. For reactions of the mercurous ion the titration gave the concentration directly. Tests showed that nitric acid in the concentrations which would occur under our experimental conditions had no effect on the titrations provided that these were carried out within 10 min. of addition of the reagents. Formic acid was also without effect on the titration. Mercuric-ion concentrations in stock solutions were determined by titration with potassium thiocyanate.

Kinetic Measurements.—All experiments were carried out at a constant ionic strength of 1·0 and in a pH range of 0—1·7 (calculated from the nitric acid content of the solutions), these conditions being obtained by the use of nitric acid–potassium nitrate solutions. Approx. 0·1M-stock solutions of mercuric and mercurous nitrates in dilute nitric acid of known concentration were made, and the mercury salt content determined accurately by the methods described above. To prepare a reaction mixture the appropriate volume of stock mercury salt solution was diluted with water and nitric acid and potassium nitrate solutions, the mixture brought to the desired temperature in a bath thermostatically controlled to $\pm 0{\cdot}02°$, and the requisite volume of standard formic acid solution (also previously brought to bath-temp.) added to start the reaction. Samples were removed at suitable time intervals, added to acidified potassium iodide and potassium iodate solution, and immediately titrated with standard thiosulphate. In those cases where the reaction was rapid (90% complete in less than 10 min.), samples taken with a fast delivery pipette were run into 2N-sulphuric acid immersed in a freezing mixture, to stop the reaction, and titrated as soon as possible thereafter.

In all experiments a large excess of formic acid was employed, enabling the concentration of this reactant to be regarded approximately as constant.

Results.—(1) *Reaction of mercuric nitrate with formic acid.* Under all the conditions studied the reaction was found to be of first order with respect to the mercuric-ion concentration. The following tables present values of the experimentally determined first-order constant $k_e \times 10^5$ (sec.$^{-1}$). The initial concentration of mercuric ion in all experiments was 0·0096 M. The ionic strength throughout was 1·0. The hydrogen-ion concentrations in all cases were calculated from the known amounts of nitric acid in the reaction mixtures. Data for a typical run are shown in Fig. 1.

(2) *Reaction of mercurous nitrate with formic acid.* As in the previous case, the reaction was of first order with respect to mercurous-ion concentration. The tables present the experimental first-order constant $k_e \times 10^5$ (sec.$^{-1}$): initial concentration of mercurous ion 0·0101M throughout, ionic strength 1·0, hydrogen-ion concentration calculated from the amount of nitric acid. Data for a typical run are shown in Fig. 1.

Reduction of mercuric nitrate.

Formic acid concentration = 0·198 g.-mol./l.

$1/[H^+]$, (g.-ion/l.)$^{-1}$	Temperature (K) 298·1°	308·2°	318·1°	328·1°	$1/[H^+]$ (g.-ion/l.)$^{-1}$	Temperature (K) 298·1°	308·2°	318·1°	328·1°
0·66	—	4·2	—	—	11·0	—	92·2	—	—
1·03	—	9·1	—	—	17·0	29·2	115	307	780
1·29	3·8	12·6	35·9	104	24·0	—	125	—	—
2·70	—	28·3	—	—	30·0	34·0	141	384	937
5·88	15·6	57·4	159	432					

Temperature 328·1° (K)

$[H{\cdot}CO_2H]$, g.-mol./l.	$1/[H^+]$ (g.-ion/l.)$^{-1}$ 1·00	1·30	1·60	2·70	$[H{\cdot}CO_2H]$, g.-mol./l.	$1/[H^+]$ (g.-ion/l.)$^{-1}$ 1·00	1·30	1·60	2·70
0·108	—	56·8	70·1	119	0·401	—	213	260	408
0·119	49·9	—	—	—	0·602	228	289	358	543
0·199	—	123	143	215	1·002	319	—	—	—
0·319	128	—	—	—					

$1/[H^+] = 2·7$ (g.-ion/l.)$^{-1}$

$[H{\cdot}CO_2H]$, g.-mol./l.	Temperature (K) 298·1°	308·2°	318·1°	328·1°	$[H{\cdot}CO_2H]$, g.-mol./l.	Temperature (K) 298·1°	308·2°	318·1°	328·1°
0·048	—	6·5	—	—	0·492	—	57·0	—	—
0·099	—	13·4	—	—	0·595	—	—	198	—
0·108	—	—	—	119	0·602	19·3	—	—	543
0·119	4·7	15·8	50·2	—	0·695	—	71·5	—	—
0·199	—	28·3	—	215	0·982	—	89·9	—	—
0·319	11·3	39·1	120	—	0·991	—	—	262	—
0·401	—	—	—	408	1·000	25·9	—	—	—

Reduction of mercurous nitrate.

Formic acid concentration = 0·198 g.-mol./l.

$1/[H^+]$, (g.-ion/l.)$^{-1}$	Temperature (K) 308·2°	318·1°	328·1°	338·1°	$1/[H^+]$, (g.-ion/l.)$^{-1}$	Temperature (K) 308·2°	318·1°	328·1°	338·1°
0·64	0·13	—	—	—	22·9	2·45	—	—	—
2·81	0·25	—	—	—	32·1	2·98	10·2	30·0	82·2
6·35	0·60	2·31	8·17	26·9	42·1	3·76	—	—	—
12·0	1·15	—	—	—	51·3	4·25	12·5	39·3	105
20·0	—	7·16	21·1	61·6					

$1/[H^+] = 42·1$ (g.-ion./l.)$^{-1}$

$[H{\cdot}CO_2H]$, g.-mol./l.	Temperature (K) 308·2°	318·1°	328·1°	338·1°	$[H{\cdot}CO_2H]$, g.-mol./l.	Temperature (K) 308·2°	318·1°	328·1°	338·1°
0·048	1·25	4·14	11·5	30·8	0·337	5·37	—	—	—
0·103	2·24	—	—	—	0·496	6·71	—	—	—
0·159	3·28	10·9	32·3	86·4	0·501	—	20·5	67·5	178
0·199	3·76	—	—	—	0·695	7·86	—	—	—
0·300	—	16·2	50·7	137	0·991	8·91	—	—	—

Discussion.

As the reactions of both mercuric and mercurous nitrate with formic acid are of first order with respect to the mercury salt it would seem reasonable to suggest that the rate-determining processes are

$$(1)\ Hg^{++} + H{\cdot}CO_2H \xrightarrow{k_1} Hg^{+} + H{\cdot}CO{\cdot}O + H^{+}$$

$$(2)\ Hg^{+} + H{\cdot}CO_2H \xrightarrow{k_2} Hg + H{\cdot}CO{\cdot}O + H^{+}$$

respectively. The well-known existence of the mercurous ion in the double form Hg_2^{++} would not alter the observed kinetics and would merely necessitate the writing of process (2) as

$$Hg_2^{++} + H{\cdot}CO_2H \longrightarrow Hg_2^{+} + H{\cdot}CO{\cdot}O + H^{+}$$

For convenience the mercurous ion will be written as Hg^{+}, but this must not be taken to indicate that the authors consider it to exist as such.

It was obviously necessary to determine whether only undissociated formic acid molecules could undergo reaction or whether the formate ion also took part (cf. the reaction

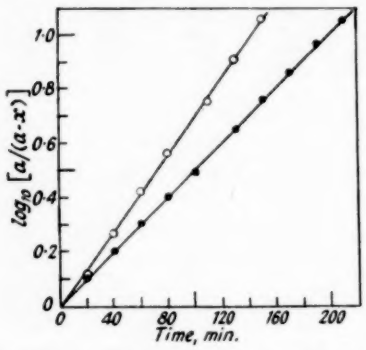
with the cobaltic ion; Bawn and White, *loc. cit.*). If the latter were the case then the reactions

$$(3)\ Hg^{++} + H{\cdot}CO{\cdot}O^- \xrightarrow{k_3} Hg^{+} + H{\cdot}CO{\cdot}O$$

$$(4)\ Hg^{+} + H{\cdot}CO{\cdot}O^- \xrightarrow{k_4} Hg + H{\cdot}CO{\cdot}O$$

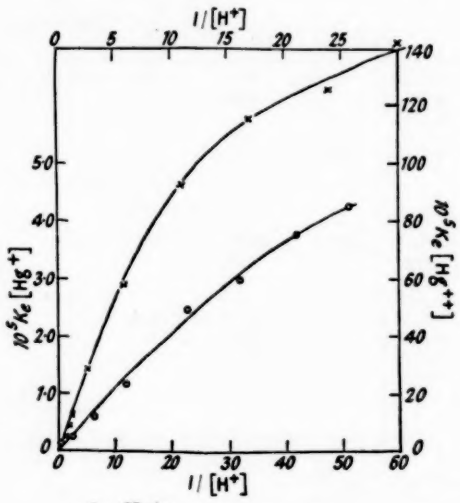
would also need to be considered. The relative importance of the reactions (1) or (2) and

FIG. 1. *Typical first-order plots.*



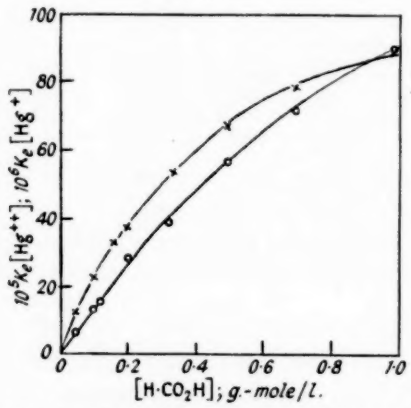
● Hg^{++}; $[H{\cdot}CO_2H] = 0{\cdot}602$ *g.-mol./l.*; $1/[H^+] = 2{\cdot}7$ *(g.-ion/l.)*$^{-1}$; $T = 25°$.
○ Hg^{+}; $[H{\cdot}CO_2H] = 0{\cdot}198$ *g.-mol./l.*; $1/[H^+] = 6{\cdot}5$ *(g.-ion/l.)*$^{-1}$; $T = 65°$.

FIG. 2.



○ Hg^{+};
× Hg^{++}; $T = 308{\cdot}2°$ (K); $[H{\cdot}CO_2H] = 0{\cdot}198$ *g.-mol./l.*

FIG. 3.



○ Hg^{++}; $T = 308{\cdot}2°$ (K); $1/[H^+] = 2{\cdot}7$ *(g.-ion/l.)*$^{-1}$.
× Hg^{+}; $T = 308{\cdot}2°$ (K); $1/[H^+] = 42{\cdot}1$ *(g.-ion/l.)*$^{-1}$.

(3) or (4) may readily be decided from the effect of hydrogen-ion concentration on the rate of reaction. The case of the mercuric ion, *i.e.*, reactions (1) and (3) being considered, the rate of reaction will be given by

$$-\mathrm{d}[Hg^{++}]/\mathrm{d}t = k_1[Hg^{++}][H{\cdot}CO_2H] + k_3[Hg^{++}][H{\cdot}CO{\cdot}O^-]$$

which, with the expression for the dissociation constant, K, of formic acid, becomes

$$-\mathrm{d}[Hg^{++}]/\mathrm{d}t = \{k_1 + k_3K/[H^+]\}[H{\cdot}CO_2H][Hg^{++}]$$

Hence, in the presence of a large excess of formic acid the experimentally determined first-order constant k_e is given by

$$k_e = \{k_1 + k_3K/[H^+]\}[H{\cdot}CO_2H]$$

and it should thus show a linear dependence on $1/[H^+]$ with a non-zero intercept at $1/[H^+] = 0$. An identical equation is obtained for the mercurous ion. Typical plots of k_e against $1/[H^+]$ are shown in Fig. 2 for both reactions. Two important facts are immediately apparent: (1) the plots are non-linear, and (2) the intercepts appear to be at the origin in both cases. The latter fact indicates at once that processes (1) and (2) can play no significant part in the reactions studied, and hence that only the formate ion is capable of reducing mercuric and mercurous ions under these conditions.

The non-linearity of the curves in Fig. 2 may be readily interpreted in terms of the hydrolysis of the mercuric and mercurous ions. It is to be expected that the two equilibria

$$Hg^{++} + H_2O \overset{K_1}{\rightleftharpoons} Hg{\cdot}OH^+ + H^+$$

$$Hg^{+} + H_2O \overset{K_2}{\rightleftharpoons} Hg{\cdot}OH + H^+$$

will be set up in the solutions. If it be conceded that the hydrolysed species are also able to react with the formate ion, then the processes

$$(5)\ Hg{\cdot}OH^+ + H{\cdot}CO{\cdot}O^- \xrightarrow{k_5} Hg{\cdot}OH + H{\cdot}CO{\cdot}O$$

$$(6)\ Hg{\cdot}OH + H{\cdot}CO{\cdot}O^- \xrightarrow{k_6} Hg{\cdot}OH' + H{\cdot}CO{\cdot}O$$

must also be considered. Again, for the mercuric ion, the rate equation becomes

$$-\mathrm{d}[Hg^{++}]/\mathrm{d}t = \{k_3K/[H^+] + k_5K_1K[H_2O][H^+]^2\}[H{\cdot}CO_2H][Hg^{++}]$$

Experimentally it is only possible to determine (by our method) the total mercuric mercury concentration $[Hg^{++}]_T$ and

$$[Hg^{++}]_T = [Hg^{++}] + [Hg{\cdot}OH^+]$$

giving for the experimentally measured rate

$$-\mathrm{d}[Hg^{++}]/\mathrm{d}t = \left\{\frac{k_3K/[H^+] + k_5K_1K[H_2O]/[H^+]^2}{(1 + K_1[H_2O]/[H^+])}\right\}[H{\cdot}CO_2H][Hg^{++}]_T$$

Thus the measured velocity constant k_e would be expected, on this basis, to show a complex, non-linear dependence on $1/[H^+]$ but to have the value zero when $1/[H^+] = 0$. An identical expression can be derived for the mercurous case. Suitable data on the hydrolysis of mercuric and mercurous salts are not yet available and hence the validity of the expression just derived cannot be further tested.

An examination of the dependence of the rate on formic acid concentration is shown in Fig. 3, where values of k_e determined at a constant hydrogen-ion concentration are plotted against formic acid concentration. It will be seen that with both the mercuric and the mercurous ion reaction the dependence is non-linear, although in the case of the mercuric ion it is approximately so up to a value of *ca.* 0·2M. However, if k_e is plotted against the square-root of the formic acid concentration then a linear relationship is found at concentrations above 0·2M. This relationship is both surprising and interesting. Such behaviour could readily be accounted for if extensive dimerisation of the formic acid occurred in aqueous solution, but all available evidence militates against this view.

Although we are unable, at present, to give a complete interpretation of our results, it does at least appear that they are not inconsistent with the occurrence of the various rate-determining steps which we have postulated. It is hoped to give a more satisfactory interpretation when further data on the hydrolysis of the mercury salts and on the state of formic acid in aqueous electrolyte solutions have been obtained.

The authors thank Professor C. E. H. Bawn for his interest in this work.

THE UNIVERSITY, LIVERPOOL. [*Received, July 23rd*, 1951.]

23. *Cobaltic Acetate. Part I.*

By J. A. Sharp and A. G. White.

This paper presents some preliminary results obtained in a reinvestigation of the preparation and properties of cobaltic acetate. The substance was prepared by an electrolytic method and was found to be readily decomposed in aqueous solutions by dilute mineral acid, cobaltous acetate being produced. The behaviour of solutions of the compound in water, alcohol, and acetic acid on treatment with ion-exchange resins has been examined. Measurements have been made of the molecular weight, conductivity, absorption spectrum (visible and ultra-violet), and magnetic susceptibilities of a number of specimens. The structure of the compound is discussed in the light of the results obtained.

Little is known of the substance which has been named cobaltic acetate, the only reported work on this compound being that of Copaux (*Ann. Chim. Phys.*, 1905, **6**, 548), Schall and Markgraff (*Trans. Amer. Electrochem. Soc.*, 1924, **45**, 161), and Schall and Thieme-Wiedtmarckter (*Z. Elektrochem.*, 1929, **35**, 337). The last two papers present contradictory results, the earlier communication claiming preparation of a compound with the analysis and molecular weight required for $Co(OAc)_3$, whereas the later paper claimed preparation of a material with analysis corresponding to $Co(OAc)_3$ but with a molecular weight corresponding to $Co_3(OAc)_9$. Further, the results obtained by Copaux were quite different from either of these claims, all his products being found to contain cobalt(II), and, in fact, it is doubtful whether Copaux isolated anything except a crude product containing much unchanged cobaltous acetate. For these reasons, and also because of the possible use of cobaltic acetate as an oxidising agent in non-aqueous media, the substance appeared worthy of reinvestigation.

The methods which have been suggested for the preparation of cobaltic acetate have all been based on either (*a*) reaction of cobaltic sulphate with an acetate or (*b*) oxidation of cobaltous acetate. Of these the electrolytic oxidation method described by Schall and Thieme-Wiedtmarckter (*loc. cit.*) was selected as apparently the most satisfactory, particularly from the point of view of attempting to isolate the product. By the method of purification used by the earlier workers, namely, extraction of the crude product with a mixture of acetic acid and light petroleum (about which no details were given), we could not obtain specimens free from cobalt(II). This method was therefore discarded; attempts to purify the compound by extraction from aqueous solutions with an organic solvent had to be abandoned because no solvent could be found which was capable of extracting the material from water.

The method of purification finally used was based on the observation that solutions of the oxidation product in acetic acid did not appear to undergo ion-exchange with cation-exchange resins whereas, under similar conditions, cobalt(II) was readily removed from solution.

A number of properties of the material prepared have been examined in an endeavour to throw some light on the structure of this curious compound.

Experimental

Preparation of Cobaltic Acetate.—A saturated solution of cobaltous acetate tetrahydrate in glacial acetic acid to which had been added 2% (v/v) of water was electrolysed in a divided cell. The anode was a platinum sheet of 40 sq. cm. area and the cathode, contained in a porous pot, was a sheet of copper of approximately the same area. The cell was cooled by immersion in a large beaker through which cold water flowed continuously. The progress of the electrolysis was followed by removing 2-ml. portions of the anolyte, adding potassium iodide, and determining the amount of iodine liberated. In some preliminary experiments the effects of temperature and current density on the current efficiency were examined. Change of temperature

between 10° and 20° was without effect, but the influence of current density was profound. The current efficiency at 100 min. fell from 66% at a c.d. of 13·7 amp./sq. m. to 2·1% at a c.d. of 100 amp./sq. m. It was thus evident that a low current density and prolonged electrolysis would be necessary. All preparations were therefore carried out at a c.d. of ~20 amp./sq. m., the volume of anolyte being 500 ml., and the duration of the electrolysis 20 hours. The temperature was kept at 10—20° during electrolysis.

After electrolysis, the anolyte was passed slowly (*ca.* 3 ml./min.) through a 2·5 × 30 cm. column of the ion-exchange resin Zeo-Karb H1. The column was freshly regenerated to the hydrogen form, according to the manufacturers' instructions, before each run and then well washed with glacial acetic acid. The dark green oxidation product passed through the column, which was washed with glacial acetic acid until the effluent was no longer green. The acetic acid solution was then evaporated *in vacuo*, at *ca.* 35°, to a syrup; this was transferred to a dish and kept in a vacuum-desiccator over phosphoric oxide until a dry product was obtained. The material thus produced was an almost black powder and will be subsequently referred to as the crude product.

Analytical Methods.—To determine Co(III), the solid compound (or a solution) was added to an excess of standard acidified ferrous ammonium sulphate solution, and the excess of ferrous iron titrated with permanganate, the end-point being determined potentiometrically. Determinations of total cobalt were made by conversion of Co(III) into Co(II) by treatment of the compound with dilute aqueous mineral acid followed by determination of the Co(II) by the volumetric oxine method.

Acetate determinations were made by distillation from solutions containing phosphoric acid, as described by Sutton (" Volumetric Analysis," 1935, p. 92) for calcium acetate.

Ion-exchange Experiments.—The ion-exchange resins used were: (1) Zeo-Karb H1, (2) Zeo-Karb 225, (3) De-Acidite " F," supplied by the Permutit Co., and (4) Amberlite IR-120-(H), supplied by B.D.H.: (1), (2), and (4) are cation-exchange resins, (2) and (4) being monofunctional (SO_3H) resins based on polystyrene, and (3) is a strongly basic anion-exchange resin.

Before use all resins were freshly regenerated, in the form required, according to the makers' instructions, and then well washed with the solvent with which they were to be used. Experiments were carried out either by shaking resin and solution in a stoppered flask, or by allowing solutions to flow through columns of the resin. The columns used were prepared in 50-ml. burettes, as this provided a convenient method of measuring bed volumes and checking flow rates.

Spectroscopy.—Absorption spectra in the range 200—1000 mμ were determined by using a Unicam S.P. 500 spectrophotometer.

Molecular-weight Determinations.—These were made by the freezing-point method. A Standard Telephones and Cables Ltd. " Thermistor " type F 2311/300 was used for temperature measurement. Temperature changes were calculated by using the approximate expression $\Delta T = k(\log R_2 - \log R_1)$, the value of the constant k being determined by separate calibration experiments. With water as solvent, urea was used for calibration. The thermistor resistance was measured by the standard bridge method and could be determined to 0·1 ohm.

Conductivity.—Conductivities were measured by means of a conventional A.C. bridge, the cell being kept at 25° ± 0·05°. The solvent was brought to bath-temperature, and a weighed quantity of solute added to a known volume; as soon as dissolution was complete, measurements could be started.

Magnetic Susceptibility.—A Curie–Chéneveau balance of the type described by Wilson (*Proc. Roy. Soc.*, 1919, *A*, **96**, 429) was employed for measurements of magnetic susceptibility. The design was modified by replacing the electromagnet by a " Magnetron "-type permanent magnet. The instrument was calibrated by using ferrous ammonium sulphate, ferric ammonium sulphate, cobaltous sulphate, cupric ammonium chloride, and potassium ferricyanide. The mass susceptibilities of these substances at room temperature were taken from International Critical Tables. All experiments were made at room temperature (17—22°).

Results.

Analysis of Solid Material.—Specimens of the crude product prepared by the method described on p. 110 had a variable Co(III) content (between 10 and 15%). The following are typical analyses.

	Co(III), %	Co(II), %	OAc, %	Unaccounted for, %
(1)	11·3	19·8	54·8	14·1
(2)	10·5	13·0	51·0	25·5

Further treatment of the crude product in acetic acid solution by ion exchange on Zeo-Karb HI (see later) gave products of an enhanced Co(III) content as the following typical figures show :

	Co(III), %	Co(II), %	OAc, %	Unaccounted for, %
(3)	17·4	12·0	39·5	31·1
(4)	16·8	8·2	50·5	25·5

On treatment of similar solutions with the ion-exchange resins Zeo-Karb 225 and Amberlite IR-120-(H), solids of much lower Co(II) content were obtained, thus :

	Co(III), %	Co(II), %	OAc, %	Unaccounted for, %		Co(III), %	Co(II), %	OAc, %	Unaccounted for, %
(5)	18·2	4·6	57·6	19·6	(7)	27·9	0·1	44·7	27·3
(6)	21·8	1·7	31·6	45·0	(8)	24·9	0·8	44·4	30·0

The significance of these figures will be discusssed later, the specimens (1)—(8) being referred to by means of these numbers in the rest of the text.

Ion-exchange Behaviour.—The behaviour of the crude product towards various ion-exchange resins has been examined in water, acetic acid, and ethyl alcohol. In all three solvents, Co(II) was found to be readily removed from cobaltous acetate solutions by the three cation exchangers in their hydrogen, sodium, and potassium forms. The Co(II) could readily be recovered from the resins by treatment with 4N-sulphuric acid. There was also found to be a slight uptake of Co(II) when cobaltous acetate solutions were run through the anion exchanger in its chloride form. The Co(II) thus taken up was removed on treatment with aqueous sodium chloride.

Prolonged contact of solutions of the crude product in any of the three solvents with any of the three cation exchangers was found to lead to a decrease in Co(III) content, and ultimately its complete disappearance. However, treatment of the exchangers, after these experiments, with sodium or potassium salt solutions or dilute mineral acid solutions failed to recover from the resin anything except Co(II). It must therefore be concluded that in contact with these resins Co(III) is reduced to Co(II). The rate of this decomposition depends markedly on the solvent and exchanger and on whether the latter is in the hydrogen or some other form. In general, reduction was rapid in aqueous solutions and much slower in the other two solvents; further, the hydrogen form of the resins was found to promote more rapid reduction than did the sodium form. The resins themselves also varied widely in their power to bring about this change, the relative rates of reduction being Zeo-Karb HI $\gg$ Amberlite IR-120-(H) > Zeo-Karb 225. The readiness with which the first of these resins causes reduction when compared with the other two is understandable, for the first is a phenolic type while the others are based on a sulphonated polystyrene and hence would be expected to be very resistant to oxidation.

The behaviour of both anolyte and solutions of the crude product on ion-exchange treatment, however, showed some unexpected features. For instance, in an experiment in which the anolyte from an electrolysis was allowed to flow through the " preparative " column of Zeo-Karb HI, the following results were obtained, Co_t representing the total cobalt :

	Anolyte	After 1 run through column	After 2nd run through column
Co_t, M/100	8·0	1·78	0·57
Co(III), M/100	1·47	0·88	0·29
$Co(III)/Co_t$	0·018	0·49	0·50

It will be seen that, although the first ion-exchange treatment brought about a great reduction in the Co(II) content, a second treatment under identical conditions did not cause any significant variation in the $Co(III)/Co_t$ ratio; but each treatment has caused considerable decomposition of the Co(III) compound. A similar experiment carried out with the resin Amberlite IR-120-(H) in the sodium form and a solution of the crude product in ethyl alcohol gave the following results :

	Original compound	Compound recovered after 1 treatment	2 treatments
Co(III), %	13·6	14·6	13·8
Co_t, %	23·4	21·0	23·3
$Co(III)/Co_t$	0·58	0·69	0·59

Repetition of the experiment with water as solvent, again with the sodium form of the resin, gave : Initial compound, $Co(III)/Co_t$ 0·58; after one ion-exchange run the solution had $Co(III)/Co_t$ 0·9. No solid could be recovered from aqueous solutions owing to rapid hydrolysis when attempts were made to remove the solvent by vacuum-distillation.

In contrast to this behaviour, when Zeo-Karb 225 or Amberlite IR-120-(H), in the hydrogen form, is used with acetic acid as solvent there appears to be no barrier to the eventual removal of all the Co(II) from solutions of the crude product, as the following results demonstrate :

	Co(III), %, in : original material	solid recovered	
Amberlite IR-120-(H), hydrogen form	12·3	23·5	
	13·0	19·1	
	13·0 *	21·8	[Co(II), 1·7; OAc, 31·6%]
	14·0	20·9	
Zeo-Karb 225, hydrogen form	17·0	24·8	
	16·0 †	27·9	[Co(II), 0·1; OAc, 44·7%]
	12·0 ‡	17·3	[Co(II), 12; OAc, 39·5%]
	11·0	18·2	[Co(II), 4·6; OAc, 57·6%]

* Two runs through column; all others, one. † Resin shaken with solution in flask for 6 hrs.
‡ Resin shaken with solution in flask for 24 hrs.

All these experiments were performed by allowing an acetic acid solution (40 ml.) of crude product (1·5 g.) to flow through a 50-ml. bed of resin at *ca.* 0·3 ml./min. In all experiments the Co(II) present in the original solution was <20% of the amount required to saturate the resin.

The behaviour of solutions of the crude product on treatment with the anion-exchange resin De-Acidite " F " yields further information. With the resin in the hydroxide form, passage of an aqueous solution of the crude product through a column of the resin gave a colloidal solution of cobaltic hydroxide. With the resin in the chloride form, an aqueous solution again being used, the following results were obtained :

Original material, %	No. of passages	OAc content of soln. as % of original value
Co(III) 21·8 Co(II) 1·7 OAc 31·6	1	64
	2	38·5
Co(III) 19·1 OAc 41·0	5	20·5

Solutions (50 ml.) contained *ca.* 2 g. of crude material and flowed through a 50-ml. column at *ca.* 0·5 ml./min. No change could be found in the Co(III) or Co(II) content of the solutions.

Properties and Reactions of Cobaltic Acetate.—The experiments described in this section were for the most part carried out with crude material containing 15—16% of Co(III).

The solubility of cobaltic acetate in a number of solvents was examined. The compound was readily soluble in acetic acid, water, and ethyl alcohol, and soluble with some difficulty in *n*-butyl alcohol, to give in all cases a very deep green solution. Solutions in acetic acid, ethyl alcohol, and *n*-butyl alcohol appeared to be stable for several months at room temperature, but a solution in ethyl alcohol on immersion in a water-bath at 95° rapidly decomposed, giving a pink precipitate and a colourless supernatant liquid. Aqueous solutions appear to hydrolyse slowly at room temperature, a brown precipitate being formed after several days; when they are heated to 60—70°, hydrolysis and formation of a precipitate occur very rapidly.

Attempts to extract the dark green Co(III) compound by shaking its aqueous solution with *n*-butyl alcohol were completely unsuccessful.

The brown precipitate produced by hydrolysis in aqueous solutions either at room temperature or on boiling retains the full oxidising power of the original solution, for on treatment with dilute sulphuric acid containing potassium iodide the theoretical quantity of iodine is liberated. Addition of sodium hydroxide to an aqueous solution also gives a brown precipitate which retains the full oxidising power and is presumably cobaltic hydroxide.

Solutions in water, acetic acid, or ethyl alcohol slowly liberate iodine from potassium iodide, the reaction taking several hours to go to completion. If the solutions are acidified, reaction is complete in a few minutes. Solutions in these solvents also rapidly oxidise acidified ferrous sulphate solutions, the Co(III) reverting to Co(II).

Addition of ethylenediamine to an aqueous solution of the compound gives a bright red colour, whereas addition of the same reagent to cobaltous acetate solution gives a brown solution which slowly becomes bright red. The addition of ammonia (*d* 0·88) to an acetic acid solution gives a bright red colour; with an aqueous solution a brown colloidal precipitate is obtained.

Aqueous solutions of the compound are rapidly decomposed on addition of mineral acid,

the colour changing from deep green to pink. The reaction with *ca.* 2N-acid at room temperature is complete in a few minutes, but in 0·1—0·2N-acid it may take 2—3 hours.

Spectroscopy.—The visible and ultra-violet absorption spectrum of the crude product was examined in water, ethyl alcohol, and acetic acid as solvents, the results being presented in Fig. 1. In Fig. 2 is shown the result of an experiment in which an excess of potassium oxalate was added to an aqueous solution of the crude product, the absorption spectrum being recorded before, and 5—10 min. after, the addition. Included in Fig. 2 is the absorption spectrum of potassium tetraoxalato-μ-dihydroxodicobalt(III), prepared by Percival and Wardlaw's method (*J.*, 1929, 2628), and it will be noticed that after addition of the potassium oxalate the absorption spectrum of the cobalt(III) acetate becomes identical with that of the oxalato-compound.

FIG. 1.

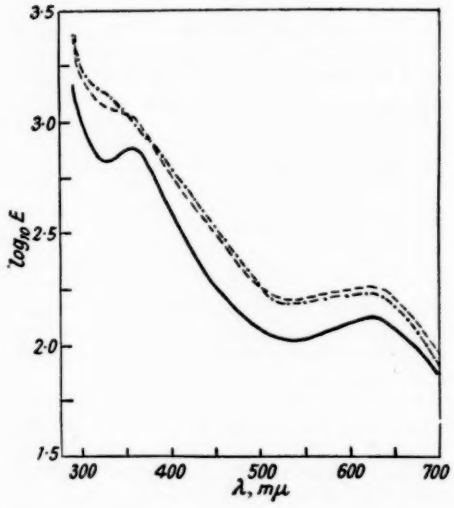


FIG. 2.

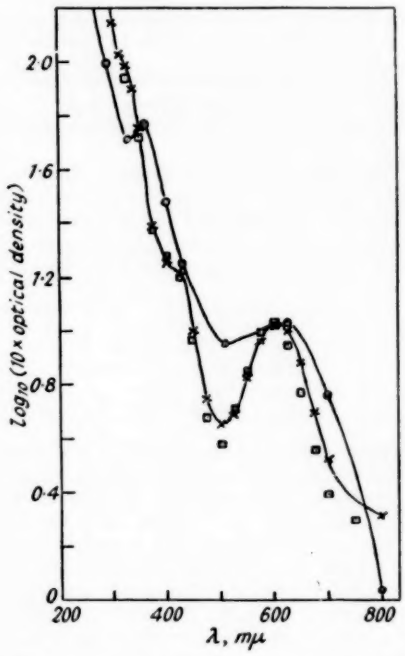


FIG. 1. *Absorption spectra of cobalt(III) acetate in :* ——, *water ;* — . —, *ethyl alcohol ;* — — — *acetic acid. The extinction coefficient* E *is calculated for* 1 *g.-atom of* Co(III) *per litre.*

FIG. 2. *Absorption spectra of :* ⊙ *cobalt(III) acetate;* ⊡ *cobalt(III) acetate* 5—10 *min. after addition of potassium oxalate ;* × *potassium tetraoxalato-μ-dihydroxodicobalt(III) ; all in water. The optical density was recorded with a* 1*-cm. cell, both solutions being adjusted to contain* $7{\cdot}75 \times 10^{-3}$ *g.-atom of* Co(III) *per litre.*

Molecular weight and conductivity. Experiments were carried out on two specimens (Nos. 5 and 6) obtained by further ion-exchange treatment of the crude product, in which the molecular weight and, in one case, the conductivity, were determined at various intervals after the solutions had been prepared. The results are presented in the following tables :

Specimen (6) : 8·04 g./1000 g. of H_2O		Specimen (5) : 14·6 g./1000 g. of H_2O		Specimen (5) : 4·14 g./1000 g. of H_2O	
Age of soln., min.	Apparent *M*	Age of soln., min.	Apparent *M*	Age of soln., min.	Apparent *M*
34	201	62	112	18	140
67	182	1170	99	44	133
165	169	4200	94	116	127
1460	135			172	124
1670	129			1320	123

Specimen (6) was used for all the following conductivity measurements, where κ is recorded in units mho/g.-ion of Co(III)/ml. and concentration in terms of g.-ions of Co(III)/l.

Concn.	308×10^{-5}		213×10^{-5}		532×10^{-6}				380×10^{-5}	
Age of soln. (min.)	9	1560	27	1200	21	1200	1·4	8·9	34·0	170·0
κ	61·5	83·7	85·5	92·0	100·6	107·0	58·1	71·1	80·7	84·4

Magnetic susceptibility. The magnetic susceptibilities of specimens (1), (2), (3), (5), (7), and (8) have been determined. In all cases a fairly large paramagnetic susceptibility was found, the results being presented in the following table:

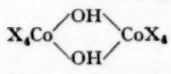
Specimen	$Co(OAc)_2,4H_2O$	(1)	(2)	(3)	(5)	(7)	(8)
$\chi \times 10^6$, c.g.s.u./g.	48·0	42·7	36·5	37·7	24·2	15·3	9·5
$\chi \times 10^3$, c.g.s.u./g.-atom of Co(III) *	—	1·39	5·73	4·57	4·83	3·19	1·87

* Corrected for Co(II), see p. 116.

Discussion

The analytical results for the specimens of cobalt(III) acetate so far obtained demonstrate clearly that not all of the compound can be accounted for in terms of Co(III), Co(II), and OAc. The discrepancy is 14—45% depending on the specimen examined. Only three samples have so far been produced in which the Co(II) content is low enough for the material to be regarded as substantially free from cobalt(II) acetate. These are Nos. (6), (7), and (8) and in none of these cases does the OAc : Co(III) ratio have the value required by $[Co(OAc)_3]_z$, and neither are the analyses of the specimens concordant. Treatment of both (5) and (6) with an excess of standard sodium hydroxide has shown that 3·09 and 2·95 equivalents, respectively, are required for precipitation of 1 atom of cobalt.

The readiness with which the compound decomposes in acid solution, the similarity of its absorption spectrum to that of potassium tetraoxalato-μ-dihydroxodicobalt(III), and the ready production of the spectrum of the latter on addition of potassium oxalate seem to indicate a close structural similarity between the two. If this hypothesis is correct, it is not improbable that cobalt(III) acetate is a binuclear complex containing the μ-dihydroxo-bridge grouping, as inset. A number of compounds of this type are already known (Werner, *Annalen*, 1910, **375**, 1; Percival and Wardlaw, *loc. cit.*) and all show a breakdown of the hydroxo-bridge on treatment with mineral acid.



The determinations of molecular weight and conductivity show that the compound reacts readily with water. Although its reactions show that hydrolysis to $Co(OH)_3$ will take place if the compound is left in water for several days, yet the rapidity with which the molecular weight falls and the conductivity increases seem to indicate the occurrence of a rapid aquotisation reaction rather than hydrolysis. This would imply great lability of the acetate groupings, and in this connection support is given by the ready production of the oxalato-compound spectrum on treatment with potassium oxalate, the rapid production of a red colour with ethylenediamine in water or ammonia in acetic acid, and the ready removal of acetate from the complex on treatment with the chloride form of an anion exchanger. Further, Werner (*loc. cit.*, p. 119) found that an acetato-group in a binuclear dihydroxy-bridged complex was readily replaced, provided that it was not acting as a bridge, by treatment with salt solutions.

The nature of that part of the compound which has not been accounted for by analysis remains obscure. If a hydroxo-bridged binuclear structure be accepted, then Co(III), Co(II), and OAc would not in any case be expected to account for the whole of the material, and, as the electrolyte used in the preparation contained 2% (v/v) of water, the presence of H_2O or OH groupings in the compound is not unlikely. However, there is some ground for the belief that a further organic grouping may occur. The ready reversion of the Co(III) to the Co(II) state in acid solution implies that either the solvent (H_2O) or some part of the compound itself is oxidised. No gas evolution can be detected, and in acid solution the reaction of the cobaltic ion with water is known to be slow (*e.g.*, Bawn and White, *J.*, 1951, 331); hence an attack on the acetate groupings appears more likely. The experimental evidence, however, does not bear out this supposition. In parallel experiments with specimen (5), acetate has been determined by (i) the normal method

and (ii) after reduction of the Co(III) with ferrous ammonium sulphate solution, and in both cases the same value is obtained. If the acetate were oxidised during the acid decomposition of the compound, then the two determinations would be expected to yield different values.

After decomposition with mineral acid in aqueous solution and precipitation of cobalt with sodium hydroxide, the solution gives a green fluorescence with β-naphthol in concentrated sulphuric acid, the intensity increasing on heating. This coloration is specific for glycollic and tartaric acids, although the latter does not give a positive test in the cold. If the compound is decomposed by treatment with ferrous salt solution and the metals are precipitated with sodium hydroxide solution before the β-naphthol test is applied, a green fluorescence is again obtained. This evidence suggests that glycollic acid may be present in the initial material, and in this connection it is significant that Schall and Thieme-Wiedtmarckter (*loc. cit.*) reported glycollic acid as a by-product of the electrolytic oxidation by which cobalt(III) acetate was prepared. The addition of sodium glycollate to acidified aqueous solutions of the compound was found to cause a marked increase in the rate of decomposition, and this indicates that glycollate, if present, would readily be oxidised.

If the compound contains a dihydroxo-bridge grouping, only 2 equivalents of sodium hydroxide per atom of cobalt should be required for precipitation of $Co(OH)_3$, whereas with samples (5) and (8) *ca.* 3 equivalents are required. As, in (8), the OAc/Co ratio is 1·7, then at least 1 equivalent of some other acid must be present, and in the case of (5) with OAc/Co *ca.* 2·5, then 0·5 equivalent of other acid is required per cobalt atom. This acid could presumably be glycollic acid, which might act as a chelating agent. The analytical results and the ready lability of the acetate (or other) groupings as indicated by the molecular weight, conductivity, and experiments with anion exchangers make it very doubtful whether any single pure compound has yet been isolated. Further, the difficulty, in some cases, of removing all the Co(II) from an apparently impure specimen by treatment with cation exchangers, under conditions in which free Co(II) is readily taken up, appear to favour the existence of some trinuclear compounds containing Co(II) (cf. Werner, *loc. cit.*, p. 40). For these reasons it seems possible that the so-called " cobaltic acetate " may in fact be a mixture of several multinuclear complexes of basically similar structure.

Finally, the measurements of magnetic susceptibility must be considered. These results are difficult to interpret, as most of the specimens contain considerable quantities of Co(II) the state of which is uncertain. However, if it is assumed that this is present as cobaltous acetate, then a deduction can be made from the observed value, based on the susceptibility found for cobaltous acetate, to allow for this. This has been done in the third line of the table in which these results were presented, and all the specimens are still found to possess a large paramagnetic susceptibility. That this paramagnetism is not a property of the cobalt(II) is clearly demonstrated by the values for (7) and (8) which contain virtually no cobalt(II). The magnetic properties of some binuclear complexes of cobalt(III) have been examined previously (Rosenbohm, *Z. physikal. Chem.*, 1919, **93**, 693; Berkman and Zocher, *ibid.*, 1926, **124**, 318) and all have been found to be diamagnetic as would be expected from the structures proposed. The only binuclear compounds of cobalt so far shown to possess paramagnetic properties are the μ-peroxo-cobalt(IV)–cobalt(III) complexes (Malatesta, *Gazzetta*, 1942, **72**, 287). The presence of Co(IV) in our samples seems unlikely in view of the facts (i) that Co(IV) has been found only in binuclear complexes with Co(III) and (ii) that these complexes all contain a peroxo-bridge (Sidgwick, "The Chemical Elements," Oxford, 1950, p. 1420). These measurements, although their interpretation must be treated with reserve, do seem to cast doubt on the validity of the postulate of a μ-dihydroxo-grouping in cobalt(III) acetate, although this might still be possible if the Co–OAc bonding possessed a largely ionic character.

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THE UNIVERSITY, LIVERPOOL. [*Received, July 23rd*, 1951.]

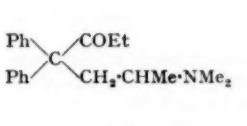
24. *Fluorene Derivatives related to Amidone.*

By G. M. Badger, J. W. Cook, and F. Schwarz.

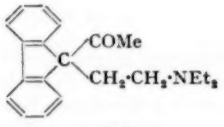
The synthesis of 9-acetyl- and 9-carbethoxy-9-diethylaminoethylfluorene is recorded.

The high analgesic potency of Amidone (I) (Bergel and Morrison, *Quart. Reviews*, 1948, **2**, 369) suggested the examination of its fluorene analogue, in which the two phenyl groups are linked in the *ortho*-positions. The experiments now described were undertaken with the object of preparing compounds of this type. They were discontinued after the publication by Ginsburg and Baizer (*J. Amer. Chem. Soc.*, 1949, **71**, 1500) of a note reporting the synthesis of the fluorene ketone probably having the same basic side-chain as Amidone.

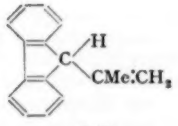
9-Cyano-9-diethylaminoethylfluorene was prepared from the potassium salt of 9-cyanofluorene and 2-chloroethyldiethylamine and was treated with methylmagnesium iodide, to give 9-acetyl-9-diethylaminoethylfluorene (II). These reactions were based on the Amidone synthesis of Schultz, Robb, and Sprague (*ibid.*, 1947, **69**, 2454). A procedure based on the alternative Amidone synthesis of Easton, Gardner, and Stevens (*ibid.*, p. 2941) gave anomalous results; the potassium salt of 9-cyanofluorene reacted with propylene oxide to give 9-*iso*propenylfluorene (III). Its structure followed from the fact that it differed from 9-*iso*propylidenefluorene but was hydrogenated to 9-*iso*propylfluorene (Maitland and Tucker, *J.*, 1929, 2559).



(I)



(II)



(III)

Ethyl 9-diethylaminoethylfluorene-9-carboxylate (cf. II) was prepared on account of its structural resemblance to some of the compounds found to have analgesic activity by Macdonald *et al.* (*Brit. J. Pharmacol.*, 1946, **1**, 4).

Experimental

9-Cyano-9-diethylaminoethylfluorene.—Potassium (3 g.), in small pieces, was added to a solution of 9-cyanofluorene (15 g.) in dry dioxan (100 c.c.), in an atmosphere of nitrogen. After 2 hours' boiling, all the potassium had reacted. The yellow potassium salt of 9-cyanofluorene crystallised. After being cooled to 50° the suspension was treated with 2-chloroethyldiethylamine (Tchoubar and Letellier-Dupré, *Bull. Soc. chim.*, 1947, **14**, 792), and the mixture then boiled for an hour. It was cooled and filtered from potassium chloride, and the dioxan removed under reduced pressure. The residual amber-coloured oil (23 g.) was dissolved in ether (100 c.c.), and the solution extracted with 1% hydrochloric acid. The aqueous extract was clarified with charcoal and basified. The free base, recovered by ether-extraction, formed a yellow oil (21·2 g.) which solidified when cooled and rubbed with a little light petroleum (b. p. 60—80°). Recrystallisation from this solvent gave *9-cyano-9-diethylaminoethylfluorene* as colourless rhombic plates, m. p. 59·5—60·5° (Found: C, 82·8; H, 7·5; N, 10·0. $C_{20}H_{22}N_2$ requires C, 82·7; H, 7·6; N, 9·6%). Its *hydrochloride* formed colourless needles (from ethanol–ethyl acetate), m. p. 205—206° (Found: C, 73·6; H, 7·0; N, 8·9. $C_{20}H_{22}N_2$,HCl requires C, 73·5; H, 7·1; N, 8·6%), and its *picrate* crystallised from ethanol in yellow needles, m. p. 157—160° (decomp.) (Found: C, 60·6; H, 4·8; N, 14·0. $C_{26}H_{25}O_7N_5$ requires C, 60·1; H, 4·8; N, 13·5%).

9-Acetyl-9-diethylaminoethylfluorene (II).—A solution of the aforesaid base (2·9 g.) in *n*-butyl ether (30 c.c.) was added dropwise to a stirred Grignard solution prepared under nitrogen from methyl iodide (5·7 g.), magnesium turnings (0·97 g.), and *n*-butyl ether (40 c.c.). Stirring was continued at 70—80° for 3 hours. The mixture was cooled and decomposed with concentrated hydrochloric acid (8 c.c.). The clear solution was decanted and the residue was treated with water (20 c.c.) and boiled. The brown oil which separated on cooling became

solid when rubbed. It was the *hydriodide* (4 g.) of (II) and crystallised from ethanol in colourless needles, m. p. 194—195° (after sintering) (Found: C, 58·5; H, 5·9; N, 3·4. $C_{21}H_{25}ON,HI$ requires C, 57·9; H, 6·0; N, 3·2%). 9-*Acetyl*-9-*diethylaminoethylfluorene* (II), extracted with ether after basification of the hydriodide solution, formed a colourless highly refractive oil, b. p. 153—154°/0·4 mm. (Found: C, 81·6; H, 7·7; N, 4·4. $C_{21}H_{25}ON$ requires C, 82·0; H, 8·2; N, 4·6%). The *hydrochloride*, prepared with ethanolic hydrogen chloride, formed colourless needles (from ethanol–ethyl acetate), m. p. 192—193° (Found: C, 73·4; H, 7·5; N, 4·2. $C_{21}H_{25}ON,HCl$ requires C, 73·3; H, 7·6; N, 4·1%). The *oxime*, prepared from a solution of the hydriodide (0·2 g.) and hydroxylamine hydrochloride (0·07 g.) in pyridine (1 c.c.) at room temperature overnight, formed colourless plates (from aqueous ethanol), m. p. 156—157° (Found: C, 78·4; H, 7·9; N, 8·4. $C_{21}H_{26}ON_2$ requires C, 78·2; H, 8·1; N, 8·7%).

9-*Diethylaminoethylfluorene*.—This was obtained in an attempt to prepare (II) from fluorene by condensation in the presence of potassium methoxide, first with ethyl acetate and then with 2-chloroethyldiethylamine, the reactions being carried out in boiling ether. The 9-*diethylaminoethylfluorene* so obtained formed a pale yellow oil, b. p. 156—158°/0·8 mm. (Found: C, 85·3; H, 8·6; N, 4·7. $C_{19}H_{23}N$ requires C, 86·0; H, 8·7; N, 5·3%). Its *perchlorate* crystallised from ethanol in rosettes of colourless needles, m. p. 181—182·5° (Found: C, 62·0; H, 6·6; N, 3·3. $C_{19}H_{23}N,HClO_4$ requires C, 62·4; H, 6·6; N, 3·8%). The base regenerated from the pure perchlorate, when treated with ethereal hydrogen chloride, gave the *hydrochloride* which crystallised from ethanol–ethyl acetate (1 : 8) in colourless hygroscopic needles, m. p. 127—129° (Found: C, 75·1; H, 7·9; N, 4·6. $C_{19}H_{23}N,HCl$ requires C, 75·6; H, 8·0; N, 4·6%). The *methiodide*, also prepared from a sample of base purified through the perchlorate, formed colourless flakes (from ethyl acetate), m. p. 157° (Found: C, 58·8; H, 6·3; N, 3·3. $C_{20}H_{26}NI$ requires C, 59·0; H, 6·4; N, 3·4%).

The same base, identified by mixed m. p. of the 3 derivatives, was formed when a solution of 9-cyano-9-diethylaminoethylfluorene (0·5 g.) in 30% methanolic potassium hydroxide (7 c.c.) was boiled under reflux for 48 hours.

9-iso*Propenylfluorene*.—The potassium compound of 9-cyanofluorene (4·9 g.) was prepared in dioxan (40 c.c.) with sliced potassium (1 g.). Propylene oxide (6 g.) was added to the cooled suspension and the mixture was heated under gentle reflux for an hour, during which the yellow potassium salt became replaced by a fine white suspension. After cooling, the solution was filtered and then boiled with charcoal for 15 minutes. Removal of the solvent under reduced pressure yielded a brown oil which solidified when cooled (4·8 g.). Recrystallisation from methanol gave colourless plates of 9-iso*propenylfluorene*, m. p. 97—98° (Found: C, 93·0; H, 7·0. $C_{16}H_{14}$ requires C, 93·2; H, 6·8%). Microhydrogenation over palladium black led to absorption of hydrogen corresponding to one ethylenic bond. Hydrogenation of the hydrocarbon (0·1 g.) in acetic acid (10 c.c.) over palladium–charcoal (0·15 g.) gave 9-*iso*propylfluorene, m. p. 55°, alone or mixed with an authentic specimen (Maitland and Tucker, *loc. cit.*).

Ethyl 9-*Diethylaminoethylfluorene*-9-*carboxylate Hydrochloride*.—Potassium (0·23 g.), in thin slices, was added to a solution of ethyl fluorene-9-carboxylate (1·4 g.) in dry ether (5 c.c.) under nitrogen. When the vigorous reaction had subsided, dry ethanol (0·3 c.c.) was added and the mixture kept at room temperature until reaction was complete. A solution of 2-chloroethyldiethylamine (0·67 g.) in ether (5 c.c.) was then added and the whole boiled for an hour. The filtered solution was twice extracted with dilute hydrochloric acid. Basification of the aqueous extract yielded an oil which was extracted with ether, and the extract was dried (Na_2SO_4). Passage of hydrogen chloride gave a resinous precipitate (1·15 g.). This was dissolved in ethyl acetate, and a little ether added. *Ethyl* 9-*diethylaminoethylfluorene*-9-*carboxylate hydrochloride* crystallised in rhombic plates, m. p. 159—160° (Found: C, 70·2; H, 7·4; N, 4·0. $C_{22}H_{27}O_2N,HCl$ requires C, 70·7; H, 7·5; N, 3·7%). The free base, which decomposed on distillation at 0·4 mm., gave a *methiodide* which formed small colourless needles (from dioxan), m. p. 156° (Found: C, 57·0; H, 6·3; N, 3·0. $C_{23}H_{30}O_2NI$ requires C, 57·6; H, 6·3; N, 2·9%).

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25. *The Reaction between Ferric and Iodide Ions. Part I. Kinetics and Mechanism.*

By A. J. Fudge and K. W. Sykes.

Previous work on the kinetics of the reaction between ferric and iodide ions has been carried out either at varying ionic strength or in the presence of a high concentration of chloride at which ferric ions are largely converted into various ferric–chloride complexes. Considerable uncertainty arises in the interpretation of such results.

Measurements have now been made at constant ionic strength and with the ferric ion present in its simple hydrated form without appreciable complex formation. The main features of the dependence of the rate of reaction on the various concentrations may be represented by the expression

$$\text{Rate} = \frac{k_1[Fe^{3+}][I^-]^2}{1 + k_2[Fe^{2+}]/[Fe^{3+}]}$$

It is concluded that the reaction probably occurs through the intermediates FeI^{2+} and I_2^- according to the following sequence of simple stages: $Fe^{3+} + I^- = FeI^{2+}$, $FeI^{2+} + I^- = Fe^{2+} + I_2^-$, $Fe^{3+} + I_2^- = Fe^{2+} + I_2$.

The oxidation of ferric ions by iodide ions,

$$Fe^{3+} + I^- = Fe^{2+} + \tfrac{1}{2}I_2 \quad . . . . . (1)$$

presents two main features of fundamental kinetic interest. The simplicity of the stoicheiometric equation suggests that this system might well afford a favourable opportunity of determining the detailed mechanism of an electron-transfer reaction. In view of the tendency of ferric ions to associate and to form complexes with a wide variety of anions, there is also the possibility of investigating the influence of such factors. Although the reaction has been studied on a number of occasions during the last fifty years, neither of these fundamental aspects has yet been fully explored.

The first kinetic measurements were made by Schurakew (*Z. physikal. Chem.*, 1901, **38**, 353), who concluded that the reaction was of the first order with respect to ferric ion and of the second order with respect to iodide ion. There is always some uncertainty, however, about any investigation of a reaction involving multivalent ions which was carried out before the necessity of controlling the ionic strength was appreciated. Sasaki (*Z. anorg. Chem.*, 1924, **137**, 181, 291) studied the reaction extensively in the presence of 2M-sodium chloride. More recent work on the composition of solutions containing ferric and chloride ions (Rabinowitch and Stockmeyer, *J. Amer. Chem. Soc.*, 1942, **64**, 335) shows that in Sasaki's experiments the iodide ion must have been reacting predominantly with various ferric–chloride complexes rather than with the simple ferric ion. Under those conditions the reaction was found to be of the first order with respect to ferric, of between the first and second order with respect to iodide, and to be retarded by ferrous ion. Wagner (*Z. physikal. Chem.*, 1924, **113**, 261), who also worked in the presence of excess of chloride (1·5M-KCl), agreed with Sasaki that the retardation by ferrous ion should be represented by writing the denominator of the rate expression in the form $1 + k[Fe^{2+}]/[Fe^{3+}]$. This he explained in terms of competition between ferric and ferrous ions for reaction with I_2^-:

$$I_2^- + Fe^{3+} = Fe^{2+} + I_2 \quad . . . . . (2)$$
$$I_2^- + Fe^{2+} = Fe^{3+} + 2I^- \quad . . . . . (3)$$

Kiss and Bossanyi (*Z. anorg. Chem.*, 1930, **191**, 289) avoided the complication of a high concentration of chloride, but allowed the hydrogen-ion concentration and the ionic strength to vary during investigation of the order of reaction. They concluded that the relation

$$\text{initial rate} = k[Fe^{3+}][I^-]^2 \quad . . . . . (4)$$

was closely obeyed if hydrolysed ferric ion was assumed to be unreactive and allowance was made for the variation of k with the ionic strength (I) by means of the equation

$$\log_{10} k = \log_{10} k_0 - 2{\cdot}0I^{\frac{1}{2}} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (5)$$

This procedure, however, does not establish the kinetics of the reaction with any certainty; moreover, a mistake appears to have been made in the derivation of equation (5), for Brönsted's theory of the primary salt effect in dilute solution gives

$$\log_{10} k = \log_{10} k_0 - 5{\cdot}0I^{\frac{1}{2}} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (6)$$

This implies that the corrections made to the observed rate by Kiss and Bossanyi, already as much as a factor of 4, were less than half as large as they should have been. Even if equation (6) was used, no quantitative conclusions could be drawn from these data; the ionic strength ranged from 0·01 to 0·12 and was thus too high for the Debye–Hückel limiting law to be obeyed accurately by triply charged ions. Some further papers record observations on the salt effect in more concentrated solutions (Kiss and Bossanyi, *ibid.*, 1931, **198**, 102) and in mixed solvents (Kiss and Vass, *ibid.*, 1932, **206**, 192).

In view of this rather confused situation, it was decided to reinvestigate the kinetics under more favourable conditions; *i.e.*, at constant ionic strength, in the presence of sufficient acid to suppress hydrolysis of ferric ion, and in the absence of anions with any strong tendency towards complex formation. This work is described in the present paper. Part II deals with the effect of complex-forming anions in the light of current theories about the kinetic significance of ionic association. In both these investigations the main object is to make a preliminary survey of the more general aspects of the reaction; it is hoped later to study in more detail those features which are found to be of particular interest. The experimental methods are consequently the simplest which would provide results of sufficient accuracy for the present purpose.

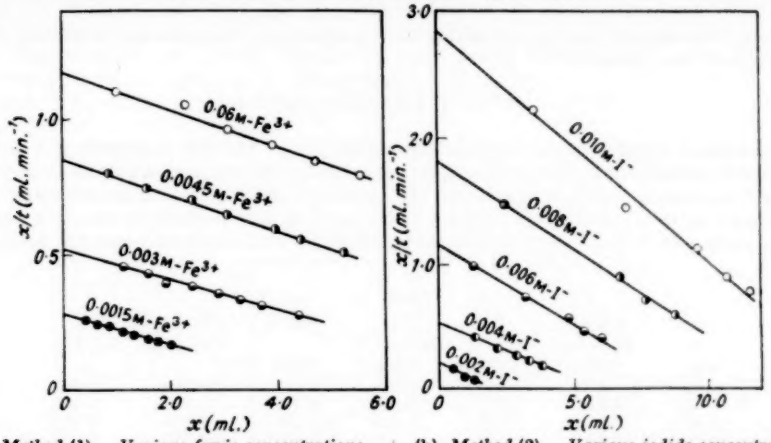
Experimental

Method.—Ferric solutions were prepared from the nitrate, nitric acid being used to control the hydrogen-ion concentration, and potassium nitrate to adjust the ionic strength. Although perchlorate is the anion with the least tendency to form complexes, the more readily available nitrate ion behaves similarly.

In previous work thiosulphate was added initially and the time required for the liberation of iodine was determined. Since under those conditions some thiosulphate may react with ferric ion, two different methods are employed here. In method (1) a slight excess of thiosulphate (x) is quickly added after the reaction has been occurring for a few minutes and the time (t) at which the iodine reappears is noted. After several successive titrations the initial rate (ml. of 0·01M-thiosulphate per min. per 110 ml. of reaction mixture) can be determined as the extrapolated value of x/t when x is zero (Fig. 1*a*). (To convert into g.-mol. $l.^{-1}$ $min.^{-1}$, multiply by $0{\cdot}91 \times 10^{-4}$.) In method (2) samples were withdrawn by pipette and diluted to stop the reaction, and the iodine determined with thiosulphate. The initial rate can be found by a similar method (Fig. 1*b*), but a larger extrapolation must be made because iodide is not being regenerated and the rate of reaction consequently decreases more rapidly Although the accuracy is also reduced by the greater dilution at which the titration is carried out, such experiments provide a useful check on the reliability of method (1). Diffuse daylight, in which all the recorded measurements are made, does not affect the rate of reaction.

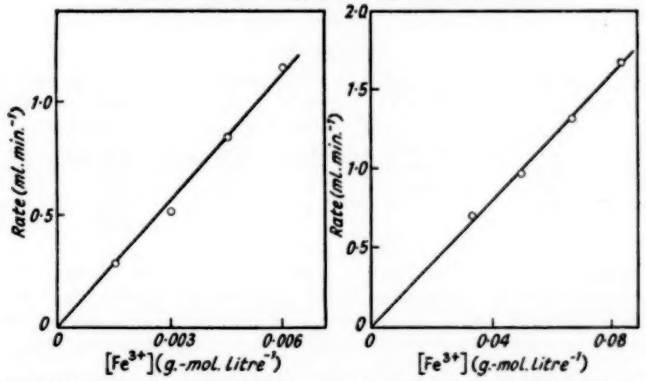
Results.—The initial rate is used to investigate the order of reaction, so that interference by the retarding ferrous ion may be avoided. Both methods show that the reaction is of the first order with respect to ferric ion and of the second order with respect to iodide ion (Figs. 2 and 3). At the hydrogen-ion concentrations of 0·009M (Figs. 2*a* and 3*a*) and 0·1M (Figs. 2*b* and 3*b*) 19% and 2%, respectively, of the total ferric ion is present as the unreactive $FeOH^{2+}$ (Part II). Sasaki's observation that in the presence of 2M-sodium chloride the reaction tends to be of first order with respect to iodide at low concentrations of the latter must be attributed to some specific effect of the chloride ion. Since Kiss and Bossanyi found the right order of reaction by the method criticised above, some fortuitous cancellation of errors must have occurred in their correction for the varying ionic strength. Evidently the underestimate of the coefficient of $I^{\frac{1}{2}}$ in equation (5) has been rather closely balanced by the known tendency

FIG. 1. *Determination of initial rate by extrapolation.*



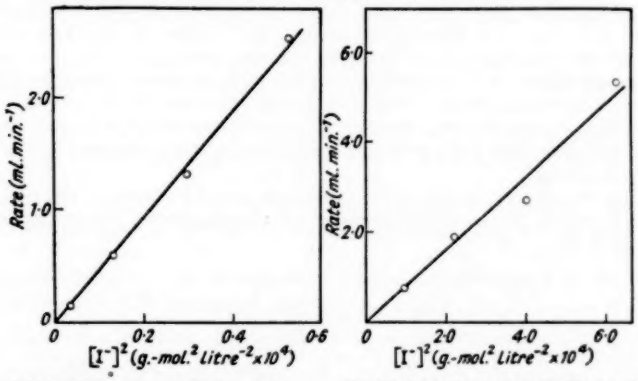
(a) *Method* (1). *Various ferric concentrations.* 19°. (b) *Method* (2). *Various iodide concentrations.* 20°.

FIG. 2. *Variation of rate with ferric concentration.*



(a) *Method* (1). 0·0036M-I^-; I = 0·0673. 19°. (b) *Method* (2). 0·002M-I^-; I = 0·602. 20°.

FIG. 3. *Variation of rate with square of iodide concentration.*



(a) *Method* (1). 0·003M-Fe^{3+}; I = 0·0673. 19°. (b) *Method* (2). 0·0017M-Fe^{3+}; I = 0·160. 20°.

of the Debye–Hückel limiting law to overestimate the deviation of activity coefficients from unity.

The retarding effect of ferrous ion having been confirmed by the addition of ferrous nitrate, a test is made of the equation

$$\text{Rate} = \frac{k_1[Fe^{3+}][I^-]^2}{1 + k_2[Fe^{2+}]/[Fe^{3+}]} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (7)$$

Table 1 analyses a typical experiment made by method (1). The first three columns show the volume of 0·01M-thiosulphate (Δx) added after successive intervals (Δt) to a mixture of volume 110 ml. and the corresponding average rates of reaction ($\Delta x/\Delta t$). The average ferric and iodide concentrations in each interval being known (cols. 4 and 5), it is possible to calculate what the rate of reaction would have been if these concentrations had remained equal to their initial

TABLE 1.

Δx, ml.	Δt, min.	$\Delta x/\Delta t$, ml./min.	$[I^-]$	$[Fe^{3+}]$ m.-mol./l.	$[Fe^{2+}]$	Corr. rate, ml./min.	R	k_2
0·00	0·00	0·283	3·636	1·515	0·000	—	—	—
0·40	1·55	0·258	3·618	1·497	0·018	0·264	0·072	6·0
0·23	1·07	0·215	3·612	1·463	0·047	0·226	0·254	8·0
0·23	1·07	0·215	3·605	1·439	0·067	0·230	0·229	4·9
0·24	1·50	0·160	3·597	1·415	0·088	0·175	0·616	9·9
0·20	1·35	0·148	3·591	1·392	0·108	0·165	0·712	9·2
0·24	1·81	0·133	3·579	1·368	0·128	0·152	0·868	9·3
0·21	1·75	0·120	3·576	1·347	0·147	0·140	1·027	9·4
0·25	2·10	0·119	3·568	1·324	0·168	0·141	1·001	7·9

TABLE 2.

Temp. 19°; I = 0·0673.			Temp. 19°; I = 0·0673.			Temp. 16°; I = 0·160.		
$[Fe^{3+}]$	$[I^-]$	k_2, mean	$[Fe^{3+}]$	$[I^-]$	k_2, mean	$[Fe^{3+}]$	$[I^-]$	k_2, mean
m.-mol./l.			m.-mol./l.			m.-mol./l.		
1·52	3·64	8·1	3·03	3·64	7·7	1·67	10·0	2·9
3·03	3·64	7·6	3·03	5·46	5·2	1·67	15·0	2·2
4·55	3·64	5·2	3·03	7·27	4·2	1·67	20·0	2·0
6·06	3·64	5·8				1·67	25·0	1·5
						1·67	30·0	1·6

values. The progressive fall of even these corrected rates (col. 7) is a measure of the retardation due to ferrous ion. Reference to the value of the equilibrium constant given by Dawson and Spivey (*J.*, 1932, 1838) proves that the contribution of the reverse reaction may be neglected. The last two columns show the magnitude of the retardation term, R, defined by the equation

$$\text{Rate} = k_1[Fe^{3+}][I^-]^2/(1 + R) \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (8)$$

and also of k_2.

Only the first figure of k_2 is significant, because the error produced in k_2 by variations of 0·01 ml. in Δx and of 1 second in Δt is 1·4. Since the average deviation of the eight k_2 values from their mean is, in fact, 1·4 although R changes by a factor of 14, Table 1 establishes the proportionality of the retardation term to the ferrous concentration. The first part of Table 2, which gives average values of k_2 for different initial ferric concentrations, supplies reasonable evidence for the inverse dependence of the retardation term on the ferric concentration.

Equation (7) also requires the retardation term to be independent of the iodide concentration, but the second and the third part of Table 2 indicate that k_2 falls somewhat as the iodide concentration is increased.

These conclusions are confirmed by the results obtained by method (2). If $a - x$, $b - x$, and x are respectively the ferric, iodide, and ferrous concentrations, the integrated form of the rate expression becomes

$$\alpha = k_1 - k_2\beta \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (9)$$

where

$$\alpha = \frac{1}{(a-b)^2 t}\left\{\frac{x(a-b)}{b(b-x)} + \ln\frac{a(b-x)}{b(a-x)}\right\} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (10)$$

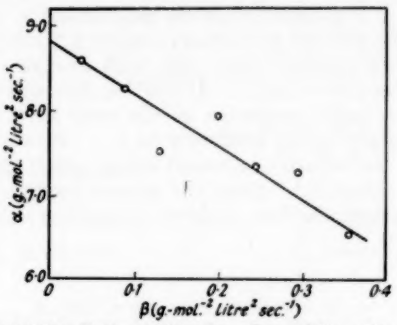
and

$$\beta = \frac{1}{(a-b)^2 t}\left\{\frac{x}{a-x} + \frac{x}{b-x} + \left(\frac{a+b}{a-b}\right)\ln\frac{a(b-x)}{b(a-x)}\right\} \quad . \quad . \quad . \quad (11)$$

Fig. 4 shows that the required linear relation between α and β is well satisfied by a typical run (0·00167M-Fe^{3+} and 0·004M-I^-); the value of 6·3 obtained for k_2 from the slope is also in good agreement with the results given in Table 2. Other k_2 values found in this way at the same iodide concentration, *viz.*, 6·8 at 0·0067M-Fe^{3+} and 4·2 at 0·0083M-Fe^{3+}, confirm the inverse proportionality of the retardation term to the ferric concentration. The iodide variation runs made by method (2) support the view that k_2 decreases as the iodide concentration is raised.

It may be concluded that equation (7), originally derived by Wagner for the reaction in the presence of 1·5M-potassium chloride, is also valid in the absence of chloride, with the exception of the dependence of k_2 upon iodide concentration noted above.

FIG. 4. *Test of equation* (9).



0·00167M-Fe^{3+}; 0·004M-I^-; $I = 0·164$. 20°.

DISCUSSION

The most probable mechanism is thought to be

$$Fe^{3+} + I^- \underset{k_2}{\overset{k_1}{\rightleftharpoons}} FeI^{2+} \quad . . . . . (12)$$

$$FeI^{2+} + I^- \underset{k_4}{\overset{k_3}{\rightleftharpoons}} Fe^{2+} + I_2^- \quad . . . . . (13)$$

$$Fe^{3+} + I_2^- \underset{k_6}{\overset{k_5}{\rightleftharpoons}} Fe^{2+} + I_2 \quad . . . . . (14)$$

The only fundamentally different possibility is to introduce free iodine atoms. No scheme of that type, however, has been found to explain the observed kinetics without arbitrary assumptions which cannot be justified in terms of general principles.*

The mechanism proposed above differs from the one originally suggested by Wagner in that the rather improbable termolecular process

$$Fe^{3+} + I^- + I^- = Fe^{2+} + I_2^- \quad . . . . . (15)$$

is replaced by two bimolecular stages. It is thus brought fully into accord with the general principle of economy of structural change in each elementary process (Hinshelwood, *J.*, 1947, 694). The successive steps now involve nothing more complicated than the association of two oppositely charged ions, the transfer of a single iodine atom, and the transfer of a single electron. Independent evidence supports the introduction of FeI^{2+} as an intermediate. $FeCl^{2+}$ and $FeBr^{2+}$ are well-known species; they have been characterised spectroscopically and the equilibrium constants for their formation determined (Rabinowitch and Stockmeyer, *loc. cit.*). Since the stability decreases on passing from the chloride to the bromide, it is reasonable to suppose that the iodide has the properties expected of a transient entity in a rapid reaction.

* The only iodine-atom mechanism consistent with equation (7) appears to be: $FeI^{3+} + I^- = FeI^+ + I$; $FeI^+ = Fe^{3+} + I^-$; $FeI^{2+} + I = Fe^{2+} + I_2$. There seems to be no general reason, however, why iodide ion should lose an electron to FeI^{2+} rather than to Fe^{3+}, or why iodine atoms should react with FeI^{2+} rather than combine with each other.

The above mechanism gives for the rate of the forward reaction

$$\text{Rate} = \frac{k_1 k_3}{k_2}[Fe^{3+}][I^-]^2 \Big/ \left(1 + \frac{k_4[Fe^{2+}]}{k_5[Fe^{3+}]} + \frac{k_3}{k_2}[I^-]\right) \quad . \quad . \quad . \quad (16)$$

This is of the same form as the observed rate expression, equation (7), provided that $k_3[I^-] \ll k_2$; *i.e.*, if the equilibrium between FeI^{2+} and its constituents is not appreciably disturbed by the further reaction of FeI^{2+} with I^-. Since ion-pair complexes are probably formed and dissociated very rapidly, the required condition is likely to be satisfied.

The variation of the ferrous retardation with the iodide concentration cannot be attributed to the last term in the denominator of equation (16), because the initial rate remains strictly proportional to the square of the iodide concentration up to the highest values investigated. If it is assumed that the mechanism is essentially unchanged, the results imply that, as the iodide concentration is raised, I_2^- reacts either more rapidly with ferric or more slowly with ferrous ions. Any such change could be due only to the association of either Fe^{3+} or Fe^{2+} with I^-. If FeI^{2+} is sufficiently more reactive than Fe^{3+}, it need not represent any large proportion of the total ferric concentration in order to make the major contribution to the oxidation of I_2^-. However unreactive FeI^+ might be compared with Fe^{2+}, the retardation would not be much affected unless half or more of the ferrous ions were associated. Since the general tendency is for ferrous ions to be less associated than ferric ions, the first explanation appears to be the more probable.

UNIVERSITY COLLEGE, SWANSEA. [*Received, July 25th,* 1951.]

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26. *The Reaction between Ferric and Iodide Ions. Part II.* The Influence of Ionic Association.*

By K. W. SYKES.

Measurements are made of the retardation of the ferric–iodide reaction as perchlorate ions are replaced, at constant ionic strength, by chloride, bromide, nitrate, and sulphate, and as the hydroxyl-ion concentration is allowed to rise.

It is expected theoretically that each of the added anions, X, will associate with the ferric ion to form an inactive complex of the type FeX. This supposition is verified for hydroxyl ions, since the equilibrium constant for the association may be evaluated from the kinetic results in reasonable agreement with independent determinations. The equilibrium constants for the formation of $FeSO_4^+$ and $FeNO_3^{2+}$, which do not appear to have been measured before, are assigned the preliminary values of $1{\cdot}5 \times 10^4$ and 10 respectively at zero ionic strength at 18°.

Neither chloride nor bromide is as strong an inhibitor as predicted from published data on their association with ferric ions. In the presence of chloride the rate of reaction is the same in the dark as in daylight; bromide is a photosensitive catalyst in daylight, but does not appreciably retard the reaction in the dark. These halide complexes must thus be supposed to oxidise iodide ions in the dark as well as in the light.

LARGE changes may occur in the rate of an ionic reaction if one of the reactants associates specifically with an ion of the opposite sign. This paper describes the important part which such phenomena play in the ferric–iodide reaction when any other anions are present except those with the least tendency towards complex formation.

Although the kinetic significance of ionic association has been appreciated for some time, only recently has a start been made on its experimental study in a systematic way. Some investigations have now been reported in which independent measurements of the

* Part I, preceding paper.

extent of association are correlated with the kinetic behaviour of the same ion-pair. Wyatt and Davies (*Trans. Faraday Soc.*, 1949, **45**, 774) have shown that CaS_2O_3 and BaS_2O_3 each react at the same rate with bromoacetate ions, but more rapidly than does $S_2O_3^{2-}$. Compared with hydroxyl ions, species of the type $BaOH^+$ do not appreciably catalyse the decomposition of diacetone alcohol or hydrolyse the carbethoxymethyltriethylammonium ion ($^+NEt_3{\cdot}CH_2{\cdot}CO_2Et$); their reactivity is indistinguishable from that of OH^-, however, in the hydrolysis of ethyl acetate (Bell and Prue, *J.*, 1949, 362; Bell and Waind, *J.*, 1950, 1979). All these apparently diverse facts may be interpreted, as indicated by Bell, in terms of the difference of electrical charge, both in magnitude and in distribution, between the transition state and the reactant ion concerned in the association. The present investigation provides an example of a system of a different charge type. Here the associating reactant, Fe^{3+}, bears a triple positive charge, while the transition state, as shown in Part I, has the composition FeI_2^+. It is to be expected that a given anion, X, will associate much more strongly with the reactant than with the transition state on account of the difference in charge. The ferric complex, FeX, though more stable than

FIG. 1. *Effect of hydrogen-ion concentration at 20°. I = 0·066.*

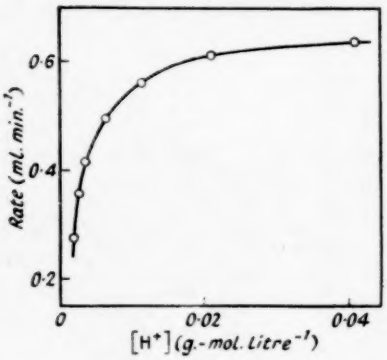
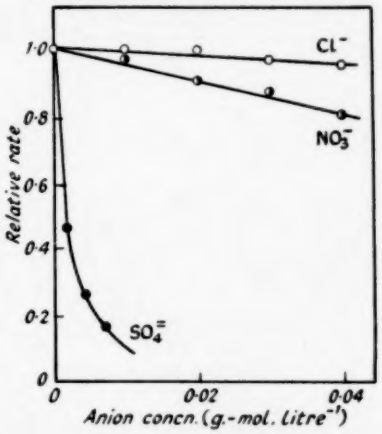


FIG. 2. *Retardation by chloride and nitrate at 20° and by sulphate at 18°. I = 0·066.*



the unassociated reactant, will thus be required to pass through essentially the same transition state and so appear to be comparatively unreactive. If the rate of reaction of FeX with I^- is negligible, the equilibrium constant for its formation from Fe^{3+} and X can be calculated from the kinetic results.

EXPERIMENTAL

Method.—The change in the rate of reaction is determined as various anions are added at constant ferric and iodide concentrations of 0·00167 and 0·005M., respectively. Since the ferric ion is supplied as ferric nitrate, and nitric acid is used to prevent hydrolysis, all solutions are 0·011M. in hydrogen ion and 0·016M. in nitrate ion unless otherwise stated. The ionic strength is controlled by perchlorate, which, in the absence of evidence to the contrary, is assumed not to associate appreciably with ferric ion. Potassium or sodium perchlorate is used in conjunction with the corresponding salt of the added anion, in order to avoid any change in the cation composition which might influence the effective iodide concentration. All the recorded rates are initial ones determined by method (1) described in Part I; they are expressed as ml. of 0·01M-thiosulphate consumed in one minute by a reaction mixture of total volume 100 ml. (To convert into g.-mol. $l.^{-1}$ $min.^{-1}$, multiply by 10^{-4}.) None of the extrapolations used to calculate the initial rates from the experimental observations is illustrated, since, with the single exception of bromide solution in daylight, they are all of the linear type shown in Fig. 1 of Part I.

Results.—Fig. 1 shows the effect of reducing the nitric acid concentration from 0·041 to 0·001M., *i.e.*, the effect of the corresponding increase of the hydroxyl-ion concentration. The nitrate-ion concentration is held constant at 0·056M. by the addition of potassium nitrate. The initial stoicheiometric hydrogen-ion concentration has been corrected by amounts not exceeding 0·0009, in order to allow for the hydrolysis of the ferric ion in the less acid solutions. It may be seen that the rate of reaction rises rapidly as the hydrogen-ion concentration is first increased, but becomes effectively independent of it above about 0·02M.

Sulphate cannot be added alone, because both the hydrogen-ion and the sulphate-ion concentrations would be seriously altered by the formation of hydrogen sulphate ions. The reaction mixture is thus made only 0·006M. in nitric acid for this particular experiment, and an SO_4^{--}–HSO_4^- buffer of the same acidity is added. On the basis of Sherrill and Noyes's data (*J. Amer. Chem. Soc.*, 1926, **48**, 1861), HSO_4^- is arranged to represent 16·7% of the total sulphate; it is unlikely that HSO_4^-, present only in this relatively small proportion, will compete appreciably with the more highly charged SO_4^{--} for association with ferric ion. Fig. 2 shows the results obtained in this way for sulphate, together with those for chloride and nitrate at the standard nitric acid concentration of 0·011M. The rates, designated relative, are expressed as fractions of those in perchlorate solution, so that the different effects of the various anions can readily be compared. A striking feature is the exceptionally strong retardation

FIG. 3. *Retardation by nitrate at 20° (◑) and by chloride in daylight at 17° (○) and in the dark at 20° (●). I = 0·426.*

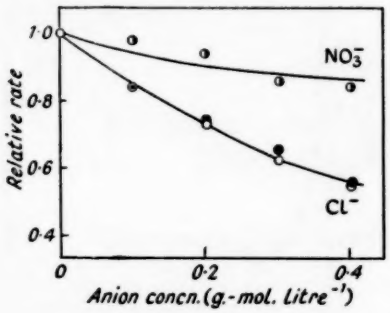
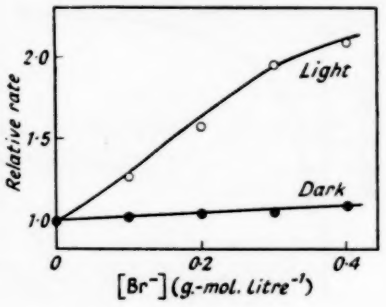


FIG. 4. *Effect of bromide concentration at 18° in daylight and in the dark. I = 0·426.*



caused by low concentrations of sulphate. The choice of perchlorate as the anion least likely to associate with ferric ion is confirmed by the slower reaction observed in the presence of nitrate. The concentration scale for the latter anion represents the amount present in addition to that originally introduced (0·016M.) as ferric nitrate and nitric acid.

The influence of chloride and nitrate at much higher concentrations and ionic strength is illustrated in Fig. 3. The similar results obtained in the dark (filled circles) and in diffuse daylight (unfilled circles) demonstrate that the chloride solutions, like all the others except the bromide, are not appreciably photosensitive under the present conditions.

Fig. 4 reveals that the reaction is strongly catalysed by bromide ions when the solution is exposed to daylight. By comparison with the results obtained in the dark, it may be seen that the effect is undoubtedly a photochemical one.

DISCUSSION

It is supposed that only unassociated ferric ions react with iodide and that their concentration is governed by equilibria of the type :

$$Fe^{3+} + H_2O = FeOH^{2+} + H^+ \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (1)$$

$$Fe^{3+} + X_i = FeX_i \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (2)$$

X_i is any anion present except hydroxyl, the effect of which is allowed for by equation (1), or perchlorate, which is assumed to remain unassociated. Since the initial rate of reaction

has been shown in Part I to be proportional to the first power of the ferric concentration, it follows that

$$\text{Rate} = \frac{k}{1 + \Sigma K_i[X_i] + K_{H^+}/[H^+]} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (3)$$

No iodide term need be included in the summation, as the fraction of the total ferric represented by FeI^{2+} may be neglected. Provided the ferric and the iodide concentration and the ionic strength are all held constant, k should not vary. K_{H^+} and K_i, which are the equilibrium constants expressed in terms of concentrations for equations (1) and (2) respectively, should also be constant at a given ionic strength. Under the present conditions, therefore, equation (3) may be tested graphically, and the equilibrium constants evaluated, by including the appropriate terms in the expression

$$\frac{1}{\text{Rate}} = \frac{1}{k} + \frac{\Sigma K_i[X_i]}{k} + \frac{K_{H^+}}{k[H^+]} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (4)$$

The Influence of Hydroxyl Ion.—Since nitrate is the only additional anion present during the experiment in which the hydroxyl-ion concentration is varied, equation (4) may be written

$$\frac{1}{\text{Rate}} = \frac{1}{k} + \frac{K_{NO_3^-}[NO_3^-]}{k} + \frac{K_{H^+}}{k[H^+]} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (5)$$

In Fig. 5 the reciprocal of the rate is plotted against the reciprocal of the hydrogen-ion concentration; the experimental points represented by unfilled circles are obtained by assuming all the hydrogen ions to be provided by the nitric acid. These points give the required linear relation in the more acid solutions, but deviate progressively as the acid concentration is reduced on account of the increasing formation of H^+ by the hydrolysis of Fe^{3+} according to equation (1).

The acid concentration, $[H^+]$, may be shown to be increased by

$$\Delta H^+ = \frac{([H^+]_0 + K')}{2}\{-1 + \sqrt{1 + 4K'[Fe^{3+}]/([H^+]_0 + K')^2}\} \quad . \quad . \quad (6)$$

where $[Fe^{3+}]$ is the total ferric concentration and $K' = K_{H^+}/(1 + K_{NO_3^-}[NO_3^-])$. According to equation (5), however, K' is precisely the quantity given by the ratio of slope to intercept in Fig. 5. It is found to be $2{\cdot}16 \times 10^{-3}$ from the straight line drawn through the experimental points obtained from the more acid solutions. On substituting this value into equation (6), the corrected points are found to be those represented by filled circles in Fig. 5. There is no significant change in the higher values of $[H^+]$, and in the middle range the agreement with equation (5) is improved. For the two solutions of least acidity, however, there are now smaller deviations in the opposite sense, but these are probably due to the formation of a small amount of the second association complex, $Fe(OH)_2^+$. The provisional determination of K' is thus confirmed.

K_{H^+} may now be evaluated as $3{\cdot}0 \times 10^{-3}$ for an ionic strength of 0·066 at 20°, since $K_{NO_3^-}$ is later found to be 6·8 under these conditions, and the nitrate concentration is 0·056M. An independent estimate of K_{H^+} may be obtained from the potentiometric work reviewed by Bray and Hershey (*J. Amer. Chem. Soc.*, 1934, **56**, 1889), whose data give $2{\cdot}65 \times 10^{-3}$ for the same ionic strength at 25°. If the heat content change is taken to be 12·3 kcal. (Rabinowitch and Stockmeyer, *J. Amer. Chem. Soc.*, 1942, **64**, 335), Bray and Hershey's value becomes $1{\cdot}9 \times 10^{-3}$ at 20°. The agreement is reasonably satisfactory in view of the probable errors in the two investigations. Since any significant reactivity of $FeOH^{2+}$ would lower the kinetic value of K_{H^+}, the results appear to warrant the conclusion that $FeOH^{2+}$ does not react appreciably with iodide.

Barb, Baxendale, George, and Hargrave (*Trans. Faraday Soc.*, 1951, **47**, 591) have recently treated in a similar way the effect of hydrogen-ion concentration on the rate at which Fe^{3+} reacts with O_2^- and HO_2^-. By two different experimental methods, they find

K_{H^+} (uncorrected for the presence of nitrate) to be 2·1 and 3·1 × 10^{-3} at 25° and an ionic strength of 0·435, whereas Bray and Hershey's data give 2·1 × 10^{-3} under those conditions. It is noteworthy that the work of these authors thus provides two further examples of $FeOH^{2+}$ being inactive, compared with Fe^{3+}, towards a singly charged negative ion.

The Influence of Sulphate Ion.—For the variation caused by sulphate the appropriate form of equation (4) is

$$\frac{1}{\text{Rate}} = \frac{1}{k} + \frac{K_{NO_3^-}[NO_3^-]}{k} + \frac{K_{H^+}}{k[H^+]} + \frac{K_{SO_4^{--}}[SO_4^{--}]}{k} \quad . \quad . \quad . \quad . \quad (7)$$

because some association with both hydroxyl and nitrate must also be taken into account. Fig. 6 shows that the predicted straight line is obtained when the reciprocal of the rate is plotted against the sulphate concentration. Small corrections have been applied for the amount of sulphate required by the observed rate of reaction to be associated with ferric ions. No allowance is made for the association of sulphate with potassium ions, since the effect of this factor will be opposed by any interaction between Fe^{3+} and HSO_4^-. From the ratio of slope to intercept, $K_{SO_4^{--}}/(1 + K_{NO_3^-}[NO_3^-] + K_{H^+}/[H^+])$ is found to be 688.

FIG. 5. *Test of equation* (5). $[H^+]$ *uncorrected* (○) *and corrected* (●) *for hydrolysis.*

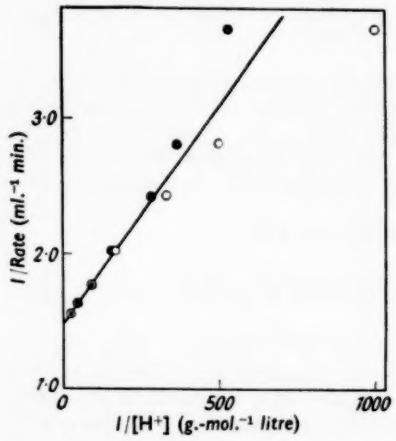
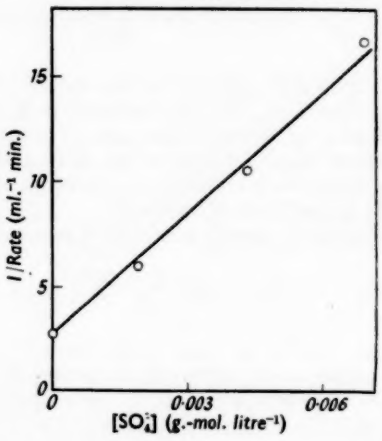


FIG. 6. *Test of equation* (7).



On insertion of the appropriate values of the nitrate and hydrogen-ion equilibrium constants and concentrations, $K_{SO_4^{--}}$ comes to 1·04 × 10^3 for an ionic strength of 0·066 at 18°.

The association of ferric and sulphate ions does not appear to have been studied previously, so it seems desirable to make a preliminary estimate from the present results of the thermodynamic equilibrium constant expressed in terms of activities. On the basis of the empirical activity coefficient equation introduced by Davies (*J.*, 1938, 2093), *viz.*,

$$-\log_{10} f = 0{\cdot}5z^2[I^{\frac{1}{2}}/(1 + I^{\frac{1}{2}}) - 0{\cdot}2I] \quad . \quad . \quad . \quad . \quad . \quad . \quad (8)$$

the product $f_{FeSO_4^+}/f_{Fe^{3+}} \cdot f_{SO_4^{--}}$ is found to be 14·0 at the experimental ionic strength. The thermodynamic equilibrium constant is thus calculated to be approximately 1·5 × 10^4 at 18°.

It appears that lanthanum is the only other triply charged metallic ion of which the association with sulphate has been studied. Davies (*J.*, 1930, 2421) has shown that solubility and conductivity data lead to 0·36 × 10^4 for the equilibrium constant for the formation of $LaSO_4^+$. It is noteworthy that this value is of the same order of magnitude as that now obtained for the corresponding ferric complex by a totally different method.

The Influence of Nitrate Ion.—By calculations similar to those already detailed $K_{NO_3^-}$ is found to be 6·8 for an ionic strength of 0·066, and 0·6 for an ionic strength of 0·426 at 20°. The consistency of these two results may be tested by calculating from each the thermodynamic equilibrium constant, using the activity coefficient function established for reactions of this type by Rabinowitch and Stockmeyer (*loc. cit.*). The values so obtained are 23 and 4, respectively; evidently more accurate experiments are required if reliable values are to be derived from rates of reaction varying by less than 20%.

Whereas the very strong retardation caused by sulphate makes it most unlikely that $FeSO_4^+$ reacts appreciably with iodide, the same cannot be said of $FeNO_3^{2+}$. It cannot be strictly established, therefore, that the results given above for $K_{NO_3^-}$ are other than lower limits. The present investigation does suggest, however, that the thermodynamic equilibrium constant for the association of ferric and nitrate ions is probably of the order of 10 at 20°. No previous estimate of this quantity seems to be available for comparison.

The Influence of Chloride and Bromide Ions.—From the experiments illustrated in Figs. 2 and 3 showing the effect of variation of chloride, K_{Cl^-} at 20° is found to be 1·7 at an ionic strength of 0·066 and 2·5 at an ionic strength of 0·426; Rabinowitch and Stockmeyer's spectroscopic work (*loc. cit.*) gives respectively 6·8 and 3·8 under those conditions. To bring the kinetic into agreement with the spectroscopic results, the decrease in the rate of reaction caused by the chloride would have to be nearly four times larger than that observed at the lower concentration and 20% larger at the higher concentration. Such differences seem to be well outside the present experimental error, so it must be concluded that the reaction between $FeCl^{2+}$ and iodide cannot be neglected, particularly at lower ionic strengths. It will be recalled that the experimental results (Fig. 3) indicate that this reaction is not a photochemical one.

Rabinowitch and Stockmeyer's data lead to a value of 0·54 for K_{Br^-} at 20° and an ionic strength of 0·426. When allowance is made for the effects of hydroxyl and nitrate, it is estimated that the highest concentration of bromide should reduce the rate of reaction by 15%. Fig. 4 shows, however, that even in the dark bromide does not retard the reaction significantly. It thus seems probable that $FeBr^{2+}$ also takes part in the thermal oxidation of iodide.

The photochemical oxidation observed in bromide solutions exposed to daylight may be attributed to the electron-transfer excitation discussed by Evans and Uri (*Nature*, 1949, **164**, 404):

$$Fe^{3+}\ Br^- \overset{h\nu}{\rightleftharpoons} Fe^{2+}\ Br \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (9)$$

Presumably the bromine atom removes an electron from an iodide ion and so initiates an atomic mechanism of iodine formation. A feature of the photochemical experiments is the very rapid decrease in the rate of reaction during the course of a run, a trend which becomes particularly marked at the higher concentrations of bromide. In the dark, however, either bromide or chloride keeps the rate of reaction much more constant than it is in their absence. These observations thus indicate that the photochemical and thermal reactions of iodide with these halide complexes have somewhat different characteristics and so suggest that they might proceed by different mechanisms.

UNIVERSITY COLLEGE, SWANSEA. [*Received, July 25th*, 1951.]

27. *The Interaction of Schiff's Reagent with Deoxyribonucleic Acids.*

By W. A. Lee and A. R. Peacocke.

The intensity of colour produced when a sensitive Schiff's reagent is added to solutions of deoxyribonucleic acids which have been subjected to very mild acid or alkali treatment is found to be greater than before such treatment. The rapidity with which this change occurs and the approximate equality of the effects of acid and alkali suggest an explanation in terms of the hydrolysis of labile $C_{(1)}$–phosphate linkages, the presence of which in deoxyribonucleic acids has already been proposed (Stacey, Li, and Overend, *Nature*, 1949, **163**, 538; Overend, Stacey, and Webb, forthcoming publication).

It has been suggested (Stacey, Li, and Overend, *loc. cit.*; Overend, Stacey, and Webb, *loc. cit.*) that a small proportion of the $C_{(1)}$ atoms of 2-deoxy-D-ribose in deoxyribonucleic acids may be involved in highly labile, polymeric linkages, possibly with phosphoric acid residues, as well as in linkages to the purine and pyrimidine bases. Such phosphate linkages would be very labile to acids, as shown by Friedkin's observation (*J. Biol. Chem.*, 1950, **184**, 449) that 2-deoxy-D-ribose-1 phosphate released 50% of its phosphorus as inorganic phosphate within 10—15 minutes at pH 4 and 23°, free aldehyde being formed at the same time. The phosphates of 2-deoxy-sugars are much more labile towards alkali than are the corresponding phosphates of their normal analogues (Foster, Overend, and Stacey, *J.*, 1951, 987) and substituents are usually much more readily removed from $C_{(1)}$ than from any other position (Cori, Colowick, and Cori, *J. Biol. Chem.*, 1937, **121**, 465; Farrar, *J.*, 1949, 3131). Such compounds would therefore be expected to be hydrolysed rapidly and completely by acid or alkali according to the scheme:

$$\rangle C{-}O{-}PO + H_2O \xrightarrow[OH^-]{H^+ \text{ or}} \rangle C_{(1)}{-}OH + HO{-}PO$$

(Secondary or primary phosphoryl group, ionised according to pH.)

In this reaction the glycosidic group of the sugar would be revealed and would immediately pass into equilibrium with the straight-chain *aldehydo*-form. Overend (*J.*, 1950, 2769) has shown that many sugars give a red-violet colour with Schiff's reagent (*Annalen*, 1866, **140**, 102) when there is the possibility that they can exist in the *aldehydo*-form (probably owing to the formation of a complex; Wieland and Scheuing, *Ber.*, 1921, **54**, 2527). In particular, 2-deoxy-D-ribose derivatives gave more intense colours than did their normal analogues, especially when substitution prevented the formation of the pyranose form, as in the deoxyribonucleic acids (Brown and Lythgoe, *J.*, 1950, 1990). Ketoses, glycosides, and polyhydric alcohols have been shown to give no colour (Tobie, *Ind. Eng. Chem.*, 1942, **14**, 405; Overend, *loc. cit.*). With this reagent it should therefore be possible to detect the relatively small quantities of free aldehyde groups released from deoxyribonucleic acids by mild acid or alkaline hydrolysis of any labile $C_{(1)}$ linkages of the type suggested above.

The Effect of Acid and Alkali under Mild Conditions.—Aqueous solutions of the sodium salts of deoxyribonucleic acids from herring sperm, calf thymus gland, wheat germ, and mouse sarcoma were treated with acid or alkali, at room temperature. The solutions were neutralised and the pH adjusted to 6·7 by the addition of phosphate buffer. Schiff's reagent was added and the intensity of colour produced was measured on a Spekker absorptiometer at various time intervals after the addition. The reading showed a slow, nearly linear increase with time. From a graph showing this variation (the figure is an example) interpolations were made and the effects of the treatments with acid and alkali ascertained. The experiment was also repeated with herring sperm and thymus nucleic acids which had been dried over phosphoric oxide (110°, 30 minutes, 0·01 mm. Hg) and dialysed, and with a sample of herring sperm nucleic acid which had been irradiated with

ultrasonic waves; the results of these experiments are shown in the Table. Variations in the time of treatment of the nucleic acid with acid or alkali caused no marked difference in the results: for this reason the times of treatment are not included in the Table. The same

Nucleic acid, source and treatment	Extinction coeff. of solution	Normality of acid or alkali	Time of interpolation of Spekker readings (min.)	Increase in Spekker reading after treatment with acid	alkali
Thymus gland, T_1, undried	0·625	0·03	60	0·11	0·09
			110	0·15	0·09
Thymus gland, T_1, dried	0·615	0·03	60	0·07	0·06
			110	0·08	0·06
Thymus gland, T_2, dialysed	0·642	0·025	60	0·14	0·20
			110	0·17	0·20
Thymus gland, isolated by Signer and Schwander	0·661	0·025	60	0·11	0·10
			110	0·11	0·11
Herring roe, H_1, undried	0·639	0·025	60	0·24	0·26
			110	0·23	0·27
Herring roe, H_1, dried	0·639	0·025	60	0·12	0·11
			110	0·12	0·11
Herring roe, irradiated	0·645	0·025	60	0·15	0·17
			110	0·13	0·17
Wheat germ, undried	0·654	0·025	60	0·06	0·08
			110	0·06	0·08
Mouse sarcoma, undried	0·649	0·025	60	0·10	0·16
			110	0·10	0·17
Herring roe, H_2, dialysed	0·648	0·025	60	0·18	0·23
			110	0·18	0·23

qualitative results are obtained whether Spekker readings are interpolated at 60, 110, or 160 minutes in graphs such as those in the figure.

A quantitative comparison between the results for different nucleic acids cannot be made because of variations in the concentrations of the reagents used. However, the following qualitative conclusions may be drawn. In all experiments, treatment of the nucleic acid with acid or alkali increased the intensity of the colour with Schiff's reagent; the effects of acid and alkaline treatment were approximately the same. Drying of the nucleic acids from soft herring roe approximately halved the increase in the intensity of the Schiff's colour effected by treatment with acid or alkali. The decrease in the case of the thymus gland nucleic acid was less than this in one experiment and almost negligible in the other. Drying of the nucleic acids therefore seems to render them more stable to acid and alkali, a conclusion previously derived from titration experiments (Lee and Peacocke, *J.*, in the press). Ultrasonic irradiation of the nucleic acid from herring roe causes a similar reduction (about 60%) in the increase in intensity of Schiff's colour caused by acid or alkali treatment.

The Speed of the Reaction.—Experiments were carried out with thymus and herring sperm nucleic acids in which the time of treatment with acid or alkali was varied between 7 seconds and 30 minutes. The maximum variation of the increase in Spekker reading with time of treatment was only 7—12% of the initial increase caused by 7 seconds' treatment. This initial increase was 0·30 for two samples of nucleic acids from thymus gland (T_1) and 0·25 and 0·17 for two samples from soft herring roe (H_1 and H_3 respectively). Both reactions are apparently complete within 7—12 seconds and only small changes occurred when the nucleic acids were treated for a further period up to 30 minutes.

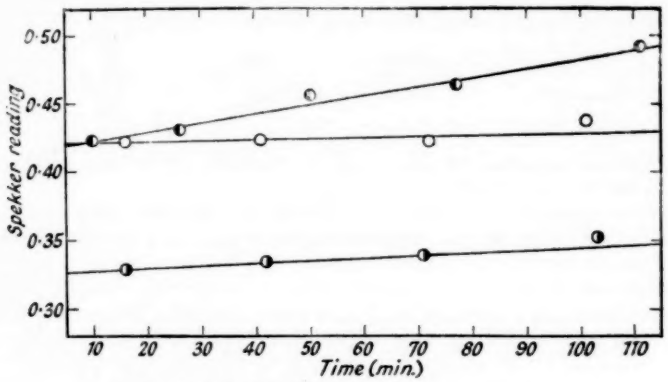
Discussion.—The results show that there is a significant increase in the intensity of the Schiff's colour given by deoxyribonucleic acids after treatment with acid or alkali under very mild conditions. Moreover, this increase is very rapid, and complete within 7—12 seconds. Since the specificity of this reagent towards the aldehyde group has been frequently demonstrated (Wieland and Scheuing, *loc. cit.*), it seems that a rapid hydrolysis of a sugar $C_{(1)}$ linkage has occurred during the treatments.

The purine–sugar and pyrimidine–sugar linkages are known to be very stable to alkali. For example, Levene and his colleagues (Levene and Bass, " The Nucleic Acids," Reinhold Publ. Co., New York, 1931, pp. 180—184) describe methods of isolating pure purine and

pyrimidine deoxyribonucleosides from an enzymic digest of thymus nucleic acid; these involve boiling of the mixture with an excess of aqueous ammonia and then allowing it to stand for 4—5 hours. It therefore seems extremely unlikely that in the very mild conditions of the experiments reported here (approximately 0·03N-alkali, room temperature for 7 seconds to 35 minutes only) hydrolysis of the bonds between the bases and the deoxy-sugar or any breakdown of the sugar ring should have occurred. Moreover, since the reaction caused by alkali is so rapid the possibility of its being due to hydrolysis of this type of linkage can be excluded. It could be explained by the presence in deoxyribonucleic acids of a small quantity of labile sugar $C_{(1)}$ linkages, as already suggested by Stacey *et al.* (*loc. cit.*) for other reasons.

Acid treatment, on the other hand, is known to hydrolyse base–sugar linkages, the bond to the purines being the least stable. Feulgen (*Z. physiol Chem.*, 1917, **101**, 296) found that the N–$C_{(1)}$ bond between the deoxy-sugar and the purine bases was completely broken by 0·15N-sulphuric acid at 80° during 40 minutes, but it has recently been shown that the deoxy-analogue of adenylic acid is converted into adenine at the rate of only

Variation of colour intensity with the time after the mixing of Schiff's reagent and thymus deoxyribonucleic acid (T_1, *undried*).



◑ *Untreated nucleic acid.*
○ *Alkali-treated nucleic acid* (pH 12, 36 *min.*).
◐ *Acid-treated nucleic acid* (pH 2, 36 *min.*).
Final pH *of all solutions* = 6·7.

2% per hour in 0·01N-hydrochloric acid at room temperature (Volkin, Khym, and Cohn, *J. Amer. Chem. Soc.*, 1951, **73**, 1533). Other hydrolyses by Volkin *et al.* indicate that the deoxy-analogue of guanylic acid would be hydrolysed at a similar rate. In our experiments with acid the conditions were the same as in the work of Volkin *et al.*, so there should only be a slow hydrolysis of the purine–sugar linkage, proceeding no further than 1% in the maximum time of acid treatment employed (*ca.* 30 minutes). Since the effect we are describing is rapid and complete well within this 30-minutes period, another explanation of the appearance of these free aldehyde groups seems necessary. There is moreover no possibility that the 2-deoxyribose ring is opening under these mild acidic conditions to give lævulaldehyde derivatives since this requires heating under pressure at 180° with methanolic hydrogen chloride (Deriaz, Stacey, Teece, and Wiggins, *J.*, 1949, 1222). The existence of a small number of labile sugar $C_{(1)}$ linkages would again account for the observations. That the same bonds are being broken by acid as by alkali is further indicated by the observation (see Table) that, within the limits of the experimental variation, the rapid initial release of aldehyde groups from the nucleic acids was approximately the same for the two treatments.

The experiments are seen provisionally to support the hypothesis of Stacey and his co-workers, but this confirmation cannot be regarded as final until the material is available

for model experiments under the same conditions on deoxyribo-nucleosides and -nucleotides and on 2-deoxy-D-ribose-1 phosphate.

Experimental

With the exception of sample H_3, the deoxyribonucleic acids are those described in earlier papers (Lee and Peacocke, *J.*, in the press). Sample H_3 was isolated from herring sperm by similar methods (Mirsky and Pollister, *J. Gen. Physiol.*, **1946**, **30**, 101). The ultrasonic-degraded herring sperm nucleic acid was obtained by irradiation of a 0·2% solution of H_1 for 95 minutes, as described previously (Lee and Peacocke, *loc. cit.*). We are indebted to Professor Signer for a sample of thymus nucleic acid isolated in his laboratory (Signer and Schwander, *Helv. Chim. Acta*, **1949**, **32**, 853).

In the experiments recorded in the Table, a solution of deoxyribonucleic acid (1 ml.) at the concentration shown in the Table was treated with hydrochloric acid or potassium hydroxide solution; 2 ml. of acid or base (0·05N) were added to the undried and dried thymus nucleic acid solutions, and 1 ml. to the other nucleic acid solutions. After 15—35 minutes the solutions were neutralised and phosphate buffer (3 ml.; 0·05M; pH 7·0) and Schiff's reagent (1 ml.) were added. The resulting solution had a pH of 6·7. The solutions were stoppered to exclude air as much as possible and the intensity of the colour produced was estimated at regular intervals by means of a Spekker absorptiometer with an Ilford 605 filter and a standard solution of a red dye. Readings were not continued beyond 3 hours, owing to the increasing importance of the gradual oxidation of the reagent by air which entered during the removal of the stopper. A control solution in which the nucleic acid was added after the hydrochloric acid, potassium hydroxide, and phosphate buffer was also prepared in order to determine the intensity of colour produced by Schiff's reagent with untreated nucleic acid.

The concentration of a nucleic acid solution was estimated by diluting it 25 times and measuring its extinction at a wave-length of 260 mμ, which is in the region of maximum absorption of the nucleic acids (for the values obtained, see col. 2 of the Table). The atomic extinction coefficients with respect to phosphorus at 260 mμ are 7700, 7500, 7800, and 7300 for calf thymus, herring sperm, mouse sarcoma, and wheat germ nucleic acids, respectively (Dr. S. Laland, private communication).

The increase in Spekker reading after acid or alkali treatment is obtained by subtracting the readings in the appropriate control experiment on untreated nucleic acid from the readings at the same interpolation time in the experiments with treated material. The results were reproducible to within $\pm$0·01 unit with the one batch of reagent.

A sensitive Schiff's reagent was prepared according to Tobie's method (*loc. cit.*) and remained colourless and stable throughout the whole investigation.

For the experiments concerned with the speed of the reactions, the following procedure was adopted. Aliquots (1·5 ml.) of a solution of the nucleic acid (0·67 mg./ml.) were treated with 0·1N-potassium hydroxide (0·5 ml.) for varying times. The solutions were neutralised with 0·01N-hydrochloric acid (0·5 ml.), and the pH was adjusted to approx. 7 by the addition of the phosphate buffer (3 ml.). The time of alkali treatment was reckoned as the time between the addition of the last drop of alkali and the last drop of acid. To each solution Schiff's reagent (1 ml.) was added and the Spekker reading noted at intervals, as above. A similar procedure, with reversal of the order of addition of acid and alkali, was used when the rate of reaction with acid was being examined.

In the latter experiments the point at which interpolations were made in the plot of Spekker reading against time did not significantly affect the results obtained. Spekker readings were again reproducible to within $\pm$0·01 unit.

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28. *Constituents of the Leaves of* Psidium guaijava, *L. Part I. Psidiolic Acid.*

By Gabra Soliman and Mahmoud Kamel Farid.

Guava leaves contain a wax, a phytosterol, and a new triterpene, psidiolic acid. It is obtainable from the alcoholic extract of the defatted leaves, and is shown to have the formula $C_{30}H_{48}O_4$ comprising two hydroxyl groups and a tertiary carboxyl group.

Psidium guaijava, L. (Myrtaceae), an arborescent shrub or small tree, which is noted for its edible fruit, is widely cultivated in Egypt. On account of their nutritive value, guava fruits have been investigated by several workers (Miller and Robbins, *Hawaii Agric. Exp. Stat. Bull.*, 1934—35, 25; Goldberg and Levy, *Nature*, 1941, **148**, 286; *J. S. Afr. Chem. Inst.*, 1942, **25**, 3; Webber, *Proc. Amer. Soc. Hort. Sci.*, 1944, **45**, 87; Quinones, Guerrant, and Dutcher, *Food Res.*, 1944, **9**, 415; Hartzler, *J. Nutrition*, 1945, **30**, 355) and shown to be rich in vitamin C. Similarly, the seed oil was examined by Kinzo, Kafuku, Hata, and Massaichi (*J. Chem. Soc., Japan*, 1934, **55**, 373), and by Varma, Godbole, and Srivatava (*Fettchem.*, 1936, **43**, 8) and its constants were determined. However, guava leaves appear to have escaped systematic investigation and the present knowledge regarding the occurrence of an essential oil is derived from Schimmel's Semi-Annual Rept., 1910, p. 123, and Klein ("Handbuch Der Pflanzenanalyse," J. Springer, 1932, Vol. II, p. 351). Recently, Schroeder (*Stud. Inst. Divi Thomae*, 1946—47, **5**, 1) studied the inhibitory action of the crude extracts of guava leaves on *Entamœba histolytica*.

Since the decoction of guava leaves is commonly used by Egyptians to alleviate cough and pulmonary disorders, we have started a systematic search for an active principle. So far, we have confirmed the presence of about 0·2% of a lemon-yellow oil, but contrary to Klein (*loc. cit.*) we were unable to prove the presence of eugenol in it. Yet the oil showed a tendency to resinify and, after ageing, its alcoholic solution gave a violet-red colour with ferric chloride.

Together with the essential oil, the petroleum extract of the leaves yielded a dark green oil which deposited on cooling an oxygen-containing wax, m. p. 82°, whereas its unsaponifiable fraction provided a crystalline phytosterol, m. p. 135—136°, the properties of which will be communicated later.

The alcoholic extract of the defatted leaves which contains an ether-soluble green resin, tannins, sugars, and an ether-insoluble brown resin proved to be a rich source for a microcrystalline substance, m. p. 252—254°. This was isolated from the ether-soluble resin by treatment with acetone and repeated crystallisation from alcohol.

Analysis indicated that this substance has the formula $C_{30}H_{48}O_4$ and contains a free carboxyl group (titration with alkali). The presence of two hydroxyl groups (primary or secondary) was indicated by the formation of a diacetate, dipropionate, and dibenzoate. Further, the acid was converted into its methyl ester by methyl sulphate or diazomethane. The ester which resisted hydrolysis by alkali was similarly converted into its dipropionate and dibenzoate. The acid gave a pale-yellow colour with tetranitromethane and the usual colour reactions which characterise triterpene acids. It appears that this compound is a new triterpene, now named psidiolic acid.

Unlike hederagenin, methyl psidiolate did not give an *iso*propylidene derivative on treatment with hydrochloric acid in presence of dry acetone (cf. Jacobs, *J. Biol. Chem.*, 1925, **63**, 621) and, in contrast to the isomeric triterpene acids, the presence of a sterically protected double bond in psidiolic acid could not be detected by means of perbenzoic acid. Attempts to prepare a lactonic isomer, the formation of which necessitates the presence of a double bond and a carboxyl group in suitable positions, by the action of hydrogen bromide in acetic acid led to resinification. The action of bromine on the acid or its methyl ester gave traces of the unchanged material as sole definite product. Further experiments are in progress.

Experimental

M. p.s are uncorrected. Microanalyses were by Drs. Weiler and Strauss, Oxford, and the analytical specimens were dehydrated at 110° over phosphoric oxide in a high vacuum.

Extraction with Light Petroleum.—The powdered leaves of *Psidium guaijava* (4 kg.), collected in summer and autumn, were extracted three times with light petroleum (b. p. 60—80°). The combined extracts yielded on concentration a dark greenish oily residue (200 g.) (cf. Abderhalden, " Biochemisches Handlexicon," J. Springer, 1911, Vol. III, p. 138) which deposited crystalline plates on cooling in the ice-chest. The deposit was freed from most of the oil and pigments by treatment with cold methanol, and purified by crystallisation from acetone and recrystallisation from benzene–light petroleum. The crystalline prisms (8 g.), m. p. 81—82°, thus obtained were recovered unchanged after being heated with acetic anhydride–pyridine, and were almost unaffected by refluxing 10% methanolic potassium hydroxide during 10 hours [Found : C, 80·4, 80·6; H, 13·6, 13·8%; *M* (Rast), 391, 413].

After separation of the wax, the greenish oil was freed from the last traces of solvent and then subjected to distillation with steam. The distillate contained a mobile lemon-yellow essential oil (0·2%) which gave a negative ferric chloride reaction. After being kept for several months, the oil became viscous and its alcoholic solution gave a violet-red colour with ferric chloride. Another sample of the essential oil was also prepared by distillation of the fresh leaves with steam, and behaved similarly.

The fatty oil was again recovered and hydrolysed with alcoholic potassium hydroxide, and the unsaponifiable fraction extracted with ether. After evaporation of ether, the orange viscous residue was treated with cold methanol, and the semi-crystalline fraction separated. The solid was purified by washing it with cold light petroleum (b. p. 40—60°), and after several crystallisations from alcohol and acetone was obtained in glistening plates, m. p. 135—136°, which yielded an acetate, m. p. 122°, and gave the colour reactions of sterols.

Extraction with Alcohol.—The defatted leaves (4 kg.) were refluxed with alcohol for 10 hours and filtered hot. This process was repeated three times, and the combined filtrates were evaporated almost to dryness under reduced pressure. The residue which contained a green resin, tannins, sugars, and a brown resin was extracted with boiling ether which dissolved only the green resin.

Psidiolic Acid.—The green resin recovered from the above-mentioned ethereal solution was treated with about 250 ml. of cold acetone, and the insoluble portion crystallised (charcoal) twice from alcohol. The discoloured product (40 g.) was further purified by washing it with acetone and ether, and finally crystallised from alcohol in spheroidal crystals, m. p. 252—254°, $[\alpha]_D^{19}$ +39·9° (*c*, 7·66 in pyridine) [Found : C, 76·5, 76·5, 75·9; H, 10·0, 10·3, 10·4; CO_2H (by titration), 9·3, 9·5, 9·5%; *M* (Rast), 450, 455. $C_{29}H_{47}O_2{\cdot}CO_2H$ requires C, 76·2; H, 10·2; CO_2H, 9·5%; *M*, 472·4]. Pure *psidiolic acid* is insoluble in light petroleum, sparingly soluble in ether, acetone, chloroform, benzene, or ethyl acetate, and is freely soluble in pyridine. It gave a yellowish colour with tetranitromethane, a reddish-violet colour with Liebermann–Burchard reagent (*Ber.*, 1885, **18**, 1803), a deep red colour with Salkowski's reagent (*Z. physiol. Chem.*, 1908, **57**, 523), and an orange ⟶ reddish ⟶ violet ⟶ brown colour with Tschügajeff's reagent (*Chem. Ztg.*, 1900, **24**, 542).

O-*Acyl Derivatives of Psidiolic Acid.*—A solution of the acid (2 g.) in pyridine (15 ml.) and acetic anhydride (15 ml.) was heated on the steam-bath for 3 hours. The product, isolated in the usual manner, crystallised from dilute alcohol in plates and recrystallised from light petroleum (b. p. 80—90°) in hexagonal prisms, m. p. 198—200° (Found : C, 73·0, 73·2; H, 9·5, 9·3. $C_{34}H_{52}O_6$ requires C, 73·3; H, 9·4%). Hydrolysis of the *diacetate* with alcoholic potassium hydroxide gave psidiolic acid, m. p. and mixed m. p. 252—254°. The *dipropionate*, similarly prepared, crystallised from dilute alcohol in needles and recrystallised from light petroleum (b. p. 80—90°) in prisms, m. p. 225—226° (Found : C, 73·8; H, 9·6. $C_{36}H_{56}O_6$ requires C, 73·9; H, 9·7%). The *dibenzoate* was prepared by heating a solution of psidiolic acid (2 g.) in pyridine (20 ml.) with benzoyl chloride (2 ml.) for 4 hours at 50°; after being kept at room temperature for 24 hours, the mixture was poured into water. The product was thoroughly washed and the resinous impurities removed by digestion with about 30 ml. of methanol. The dibenzoate crystallised from benzene–alcohol in glistening prisms, m. p. 248—250° [Found : C, 78·0, 78·2, 78·3, 78·5; H, 7·6, 7·7, 7·8, 8·0%; *M* (by titration), 666·2, 663·4. $C_{44}H_{56}O_6$ requires C, 77·6; H, 8·3%; *M*, 680·45]. Better analytical figures could not be obtained from the highly purified specimens. $[\alpha]_D^{19}$ was +0·06° (*c*, 2·76 in benzene).

Methyl Psidiolate.—Methyl sulphate (15 ml.) was gradually added, with shaking, to a

solution of psidiolic acid (5 g.) in alcohol (60 ml.) and 10% sodium hydroxide (60 ml.). After addition of more alkali and methyl sulphate, the mixture was kept at room temperature for 2 hours and the crystalline *ester* collected. It crystallised from dilute methanol and recrystallised from benzene–light petroleum (b. p. 60—80°) in glistening needles, m. p. 213—215°, after sintering at 150°, $[\alpha]_D^{19}$ +52·1° (*c*, 5·26 in ethanol) (Found : C, 76·3, 76·4; H, 10·3, 10·4; OMe, 6·7. $C_{31}H_{50}O_4$ requires C, 76·5; H, 10·4; OMe, 6·4%). The ester was recovered unchanged when its solution in dry acetone was treated with a few drops of concentrated hydrochloric acid, and it was not hydrolysed when heated with 5% alcoholic potassium hydroxide for 5 hours.

O-*Acyl Derivatives of Methyl Psidiolate.*—Methyl psidiolate reacted quite readily with acetic anhydride in presence of pyridine or fused sodium acetate, but the acetylation product was freely soluble in most of the organic solvents and did not crystallise from dilute solvents. The *dipropionate* was prepared when a solution of the ester (2 g.) in pyridine (15 ml.) and propionic anhydride (15 ml.) was kept at room temperature for 3 days. The product was isolated in the usual manner and crystallised from dilute alcohol in glistening prisms, m. p. 150° (Found : 74·6; H, 10·0; OMe, 5·9. $C_{37}H_{58}O_6$ requires C, 74·2; H, 9·8; OMe, 5·2%). The *dibenzoate* was prepared by heating a solution of the ester (1 g.) in pyridine (10 ml.) with benzoyl chloride (1·5 ml.) for 8 hours at 40°; after being kept at room temperature for 24 hours, the mixture was poured into water. The resinous product was washed free from pyridine and digested with methanol. The insoluble portion crystallised from benzene–methanol in prisms, m. p. 210°, $[\alpha]_D^{19}$ 0° (*c*, 2·89 in benzene) (Found : C, 78·0, 77·6; H, 8·5, 8·3. $C_{45}H_{58}O_6$ requires C, 77·8 ; H, 8·4%). Both the dipropionate and the dibenzoate gave the pure ester on hydrolysis with alcoholic potassium hydroxide.

Reactions for Unsaturation.—25-Ml. portions of 0·047N-solution of perbenzoic acid in chloroform were added to 0·2010, 0·1692, and 0·1803-g. portions of the dipropionate of methyl psidiolate, and the three solutions were kept at 0° for 7 days. Afterwards, the reagent which remained almost unaffected was determined in the usual manner and the ester recovered unchanged.

A solution of methyl psidiolate (2 g.) in chloroform (20 ml.) was mixed with 10 ml. of 3% bromine solution in chloroform, and after 2 hours the solvent was evaporated at room temperature. The brownish resinous residue yielded traces of the unchanged ester after crystallisation from benzene–light petroleum.

A solution of psidiolic acid (2 g.) in acetic acid (200 ml.) and water (10 ml.) was mixed with 25 ml. of 3% bromine solution in acetic acid, and after 3 hours the mixture was stirred into water and the yellowish gelatinous product collected. After two crystallisations from methanol followed by washing with acetone, it was obtained as a whitish product (0·4 g.) which recrystallised from alcohol in spheroidal crystals, m. p. 252—254°, not depressed by psidiolic acid, and gave methyl psidiolate with diazomethane.

A solution of diacetyl psidiolic acid (0·6 g.) in acetic acid (10 ml.), saturated with hydrogen bromide, was kept at room temperature for 3 days and then poured into water. The resinous product gave on crystallisation from dilute alcohol and recrystallisation from light petroleum (b. p. 80—90°) traces of the starting material.

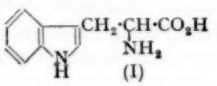
FAROUK I UNIVERSITY, MOHARRAM BEY, ALEXANDRIA. [*Received, August 24th,* 1951.]

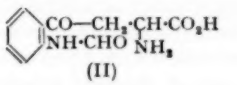
29. *The Synthesis of* N′-*Formyl*-DL-*kynurenine,* N^{α}-*Acetyl*-DL-*kynurenine and Related Compounds, and Observations on the Synthesis of Kynurenine.*

By C. E. DALGLIESH.

Exclusive formylation of the aromatic amino-group of kynurenine to give, in high yield, *N*′-formyl-DL-kynurenine (II) can be accomplished under suitable conditions. Syntheses of N^{α}-acetyl-DL-kynurenine (III), N^{α}-acetyl-*N*′-formyl-DL-kynurenine (IV; R = CHO, R′ = Ac), and related compounds are also reported, together with observations on the formylation of amino-groups and on the synthesis of DL-kynurenine

WORK in progress in these laboratories on the intermediary metabolism of tryptophan (I) (cf., *e.g.*, Dalgliesh, Knox, and Neuberger, *Nature*, 1951, **168**, 20) has made it desirable to prepare various derivatives of kynurenine which occur in, or are related to, the tryptophan metabolic chain (Dalgliesh, *Quart. Reviews*, 1951, **5**, 227). Recent work (Knox and Mehler, *J. Biol. Chem.*, 1950, **187**, 419, 431) has shown that *N*′-formylkynurenine (II) is the first identifiable intermediate in the normal biological degradation of tryptophan. Formylkynurenine has not been isolated from biological systems, in which it is normally split by the enzyme formylase to kynurenine and formic acid, but the evidence for its participation in these systems is weighty. In the laboratory it has hitherto only been prepared with some difficulty, and in low yields, by the ozonisation of tryptophan (Witkop and Graser, *Annalen*, 1944, **556**, 103; Amano, Torii, and Irritani, *Med. J. Osaka Univ.*, 1950, **2**, 45; Knox and Mehler, *loc. cit.*) and a more convenient synthesis was desirable to facilitate further investigation of its biological behaviour. Two other derivatives of kynurenine


(I)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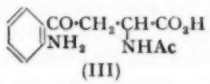
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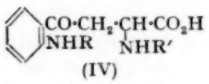
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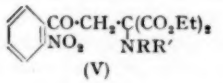
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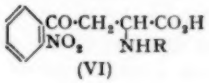
(VI)

are also of possible importance in tryptophan metabolism. N^{α}-Acetyl-L-kynurenine (III) was isolated from cultures of a *Neurospora* mutant in which there existed a genetic block preventing further metabolism of kynurenine by the normal pathway (Yanofsky and Bonner, *Proc. Nat. Acad. Sci.*, 1950, **36**, 167). It has also been detected in the urine of pyridoxine-deficient rats fed on tryptophan (Dalgliesh, Knox, and Neuberger, *loc. cit.*), and it is possible (cf. Dalgliesh, *loc. cit.*) that N^{α}-acetylkynurenine may lie on the direct tryptophan metabolic pathway in animals. If such were the case, then N^{α}-acetyl-*N*′-formylkynurenine (IV; R = CHO, R′ = Ac) might be an intermediate lying between (II) and (III). This paper describes, first, improvements in the synthesis of DL-kynurenine (IV; R = R′ = H), and, secondly, the synthesis of *N*′-formyl-DL-kynurenine (II), N^{α}-acetyl-DL-kynurenine (III), N^{α}-acetyl-*N*′-formyl-DL-kynurenine (IV; R = CHO, R′ = Ac), and of various related compounds. The preparation of optical isomers will be described in a later communication.

DL-Kynurenine was first synthesised by Butenandt and his co-workers (*Naturwiss.*, 1942, **30**, 51; *Z. physiol. Chem.*, 1943, **279**, 27) by condensing *o*-nitrophenacyl bromide with sodiophthalimidomalonic ester to give [V; RR′ = *o*-$C_6H_4(CO)_2$], which was hydrolysed to the hydrochloride of *o*-nitrophenacylglycine (α-amino-β-*o*-nitrobenzoylpropionic acid) (VI; R = H), the latter being then reduced Subsequently the synthesis was improved by the use of benzamidomalonic ester (Butenandt, Weidel, and Neckel, *ibid.*, 1944, **281**, 120) but in this case the intermediate (V; R = H, R′ = Bz), obtained as a thick red oil, was not purified. Although other synthetic routes to kynurenine are available (Sakan, *J. Chem. Soc. Japan*, 1942, **63**, 1545; Butenandt and Hellmann, *Z. Naturforsch.*, 1950,

5b, 445) the acylamidomalonate route was felt to be preferable in that it combined relative simplicity with formation of an intermediate (VI; R = H) in which the aliphatic amino-group could be modified before the aromatic amino-group was introduced. Acetamidomalonic ester is more readily available than the phthalimido- or the benzamido-analogue, and its use would avoid subsequent troublesome separations of phthalic or benzoic acid. It was found essential in using the acetamido-compound to effect the condensation of the sodio-derivative with the bromide at room temperature to avoid formation of dark by-products. Under these conditions the ester (V; R = H, R′ = Ac) was readily obtained. Although the yield of recrystallised ester was only 40%, use of this pure intermediate gave yields in the subsequent hydrolysis and hydrogenation exceeding 90% in each step, and eliminated wasteful purifications required in the previous methods. The same procedure was satisfactory with phenacyl bromide, giving, ultimately, phenacylglycine (as VI).

Conditions have been found under which it is possible to formylate the aromatic amino-group of kynurenine without affecting the aliphatic amino-group. In the first experiment kynurenine was dissolved in formic acid (98—100%), one equivalent of acetic anhydride added, and after two hours the mixture poured into ether, precipitating a cream-coloured solid. The ultra-violet spectra of *N*′-acylated kynurenines show two maxima, at about 260 and 320 mμ, whereas kynurenine and N^{α}-acylated kynurenines show maxima at about 260 and 360 mμ (cf. Knox and Mehler, *loc. cit.*). Spectroscopic examination of the reaction product showed that acylation had occurred solely on the aromatic amino-group, the low absorption at 360 mμ and absence of any sign of a peak at this point showing the absence from the product of kynurenine or a N^{α}-acylkynurenine. The product contained a free α-amino-group (ninhydrin) and was markedly water-soluble. The pH of the solution (~3·5) made it likely that the material was a salt, and addition of one equivalent of pyridine to its aqueous solution precipitated the amino-acid which analysis showed, rather surprisingly, to be *N*′-acetyl-DL-kynurenine, and not the formyl compound. The salt was the formate thereof. The free acetyl compound showed a marked tendency to form gels, but could be obtained with difficulty as crystals of hydrated material. On removal of the water at elevated temperatures the product became very hygroscopic.

When two equivalents of acetic anhydride were added to kynurenine in formic acid, diacylation occurred smoothly, and the product contained one acetyl and one formyl group. As the aromatic amino-group was found above to have been acetylated under similar conditions, the product was *N*′-acetyl-N^{α}-formyl-DL-kynurenine (IV; R = Ac, R′ = CHO). It seemed, therefore, that under the conditions used aromatic amino-groups were acetylated and aliphatic amino-groups were formylated. This was confirmed by the finding that N^{α}-acetylkynurenine (III; see below) was acetylated to give *N*′N^{α}-diacetyl-DL-kynurenine (IV; R = R′ = Ac), whereas phenacylglycine, *o*-nitrophenacylglycine, tryptophan, and phenylalanine were all formylated.

Under the same conditions anthranilic acid underwent mainly acetylation. The active agent in formylations using formic acid and acetic anhydride is presumably acetic formic anhydride, which in reactions with alcohols and aromatic amines is known to give solely formyl derivatives (Béhal, *Compt. rend.*, 1900, **128**, 1460; Hurd *et al.*, *J. Amer. Chem. Soc.*, 1939, **61**, 3355; 1946, **68**, 789). The occurrence of acetylation in the above reactions must therefore have been due to the more rapid reaction of the acetic anhydride with the aromatic amino-groups than with formic acid. In confirmation of this it was found that, if the acetic anhydride was added to the formic acid (heat is evolved), and after 30 minutes anthranilic acid was added, solely formylation occurred.

On applying these modified conditions, kynurenine gave *N*′-formyl-DL-kynurenine (II), in a yield of over 90%. As in the case of the corresponding acetyl compound, a tendency to gel formation was observed, and the compound was very hygroscopic. The ultra-violet absorption spectrum agreed with that found by Knox and Mehler (*loc. cit.*), and identity was confirmed by examination of the behaviour towards the enzyme formylase, kindly carried out by Dr. W. E. Knox: the synthetic material was hydrolysed by the enzyme at approximately seven times the rate found for the reference compound, formyl-

anthranilic acid, this ratio agreeing within experimental error with that found for formylkynurenine formed during tryptophan degradation; the rate of hydrolysis was linear with time, also a characteristic of natural formylkynurenine; *N'*-acetyl-DL-kynurenine was hydrolysed by the formylase preparation at about one-tenth of the rate of the *N'*-formyl compound, also agreeing with expectation. Further formylation of the *N'*-formyl compound gave *N'N*$^{\alpha}$-diformyl-DL-kynurenine.

N$^{\alpha}$-Acetyl-DL-kynurenine (III) was prepared by hydrogenation of *N*-acetyl-*o*-nitrophenacylglycine (VI; R = Ac). Attempted acetylation of *o*-nitrophenacylglycine with acetic anhydride alone, or with sodium acetate, pyridine, or sulphuric acid, gave only dark intractable products, and similar results were obtained on the attempted hydrolysis of the ester (V; R = H, R' = Ac) by carbonate (cf. Albertson, *J. Amer. Chem. Soc.*, 1950, **72**, 1396). However, acetylation proceeded satisfactorily under Schotten–Baumann conditions. Also prepared under these conditions was *N*-carbethoxy-*o*-nitrophenacylglycine (VI; R = CO_2Et), which was designed as an intermediate for an alternative synthesis of *N'*-formylkynurenine. Hydrogenation gave *N*$^{\alpha}$-carbethoxy-DL-kynurenine which when heated in a sealed tube with ethyl formate (Human and Mills, *J.*, 1948, 1457) gave, probably, *N*$^{\alpha}$-carbethoxy-*N'*-formyl-DL-kynurenine. In view of the success attending direct formylation of kynurenine removal of the carbethoxy-group was not attempted.

N$^{\alpha}$-Acetylkynurenine (III) was submitted to the modified formylation conditions described above, and the required *N*$^{\alpha}$-acetyl-*N'*-formyl-DL-kynurenine (IV; R = CHO, R' = Ac) was obtained.

The monoacyl derivatives of DL-kynurenine show great resistance to crystallisation. In spite of many attempts *N*$^{\alpha}$-acetyl-DL-kynurenine (III) could only be obtained as a somewhat hygroscopic glass, although analysis and paper-chromatographic examination demonstrated its homogeneity. The gel-forming tendency of *N'*-acetyl- and *N'*-formyl-DL-kynurenines has already been noted. The diacyl derivatives of kynurenine, and the nitro-compounds corresponding to kynurenine, crystallise readily.

In view of recent reports on the resolution by means of paper-chromatography of amines (Bonino and Carassiti, *Nature*, 1951, **167**, 569) and amino-acids (Kotake *et al.*, *J. Amer. Chem. Soc.*, 1951, **73**, 2973) it is interesting that DL-kynurenine is readily resolved on paper (Whatman No. 4) by use of butanol–acetic acid–water (4 : 1 : 5) (Partridge, *Nature*, 1946, **158**, 270). The D-isomer travels under these conditions at about 0·9 times the rate of the L-isomer.

Experimental

Formic acid used was 98—100% (B.D.H.). M. p.s are uncorrected.

Preparation of DL-*Kynurenine.*—Condensation of *o*-nitrophenacyl bromide and sodioacetamidomalonic ester in dry benzene under reflux resulted in a thick red-brown oil similar to that described by Butenandt *et al.* for the analogous benzamido-compound. No crystalline material could be obtained from the product, and the *o*-nitrophenacylglycine hydrochloride obtained on hydrolysis was contaminated with much tar, the removal of which was very wasteful. Hydrogenation to kynurenine did not proceed satisfactorily unless pure nitro-compound was used. The difficulties obviously proceeded from side-reactions in the initial condensation, and many other media were therefore tried. At higher temperatures results were worse, *e.g.*, in boiling xylene the whole mixture charred. The product (V; R = H, R' = Ac) was obtained crystalline in low yield after condensation in boiling alcohol, and it was then found that condensation in ethanol at room temperature occurred smoothly, the product separating from solution. Condensation at room temperature in dimethylformamide (Sheehan and Bolhoffer, *J. Amer. Chem. Soc.*, 1950, **72**, 2786) was so vigorous that a brown by-product was formed in considerable quantity, and the same occurred to a lesser extent in methanol. The following procedure has been found satisfactory.

Sodium (1·8 g.) was dissolved in ethanol (100 ml.) and ethyl acetamidomalonate (19·2 g.) added. To the solution, cooled to room temperature, was added *o*-nitrophenacyl bromide (18·6 g.), and the whole shaken overnight. The mixture was set aside for a few hours at −3°, then filtered, and the residue washed well with water and dried. Recrystallisation from ethyl acetate (with addition of light petroleum if necessary) gave *ethyl acetamido-o-nitrophenacylmalonate* (V; R = H, R' = Ac) as pale cream-coloured crystals, m. p. 124° (14·0 g., 41%) (Found: C, 53·9; H, 5·5; N, 7·7. $C_{17}H_{20}O_8N_2$ requires C, 53·7; H, 5·3; N, 7·4%).

The ester (19 g.) was refluxed with acetic acid (40 ml.) and concentrated hydrochloric acid (40 ml.) for 5 hours, a further total of 40 ml. of hydrochloric acid being added at intervals. The mixture was cooled, poured into water, any slight precipitate being removed by filtration, and extracted three times with ether. The aqueous fraction was taken to dryness in a vacuum on the water-bath, the residue taken up in boiling alcohol, and filtered, and ether added to the cooled filtrate, to give *o*-nitrophenacylglycine hydrochloride as a voluminous colourless precipitate, isolated after a few hours at 0° (yield, 13 g., 94%).

On addition of one equivalent of pyridine to a hot aqueous solution of the hydrochloride DL-*o*-*nitrophenacylglycine* (VI; R = H), white needles (from water), decomp. at 195°, separated (Found: C, 51·0; H, 4·0; N, 11·6. $C_{10}H_{10}O_5N_2$ requires C, 50·4; H, 4·2; N, 11·7%).

The nitro-hydrochloride (10 g.) in N-sulphuric acid (73 ml.) was hydrogenated over palladium–charcoal. The catalyst was removed and washed with hot water, and pyridine (9 ml.) added to the filtrate. DL-Kynurenine (3·4 g.) separated on storage and the mother-liquors on concentration gave further material (3·6 g.).

Preparation of Phenacylglycine (*α-Amino-β-benzoylpropionic Acid*).—The condensation was repeated as above, with phenacyl bromide. The reaction mixture was poured into water, and the precipitated solid hydrolysed directly, to give the *amino-acid hydrochloride*, white silky needles (from ethanol), decomp. 205°, which on addition of one equivalent of pyridine to its aqueous solution gave DL-phenacylglycine, colourless crystals (from water), decomp. 200° (Found: C, 62·1; H, 6·0; N, 7·4. Calc. for $C_{10}H_{11}O_3N$: C, 62·2; H, 5·7; N, 7·3%). This acid has been prepared by an alternative route by Fraser and Raphael (*J.*, 1950, 2245).

N′-*Acetyl*-DL-*kynurenine Formate*.—To kynurenine (1·2 g.) in formic acid (4 ml.) was added acetic anhydride (0·55 ml., 1 mol.). After 2 hours at room temperature the mixture was poured, with shaking, into ether (500 ml.), precipitating N′-*acetyl*-DL-*kynurenine formate* as an almost colourless solid (1·33 g., 78%) The ultra-violet absorption spectrum, determined in M/5-phosphate buffer, pH 7·0, diluted 1 : 10, showed maxima at 259 (ε = 9740) and 320 mμ (ε = 3080), and no sign of a peak at 360 mμ. The absence of kynurenine or an N^{α}-acylkynurenine was confirmed by paper-chromatography, which however suggested the presence of a small amount of the *N*′-formyl compound. The substance showed a great tendency to form gels, and was purified by dissolution in cold formic acid and gradual precipitation by ether at 0°. It then decomposed at 136° (Found: C, 52·8, 52·3; H, 5·4, 5·5; N, 9·7. $C_{12}H_{14}O_4N_2,CH_2O_2$ requires C, 52·7; H, 5·4; N, 9·5%). The free hydrated acetyl compound was obtained on addition of pyridine to an aqueous solution of the formate. On being dried in a vacuum at elevated temperatures the product lost water and became very hygroscopic and difficult to analyse. The product obtained was approximately a *hemihydrate* (Found: C, 55·2; H, 5·5; N, 11·0. $C_{12}H_{14}O_4N_2,\frac{1}{2}H_2O$ requires C, 55·6; H, 5·8; N, 10·8%).

N′-*Acetyl*-N^{α}-*formyl*-DL-*kynurenine* (IV; R = Ac, R′ = CHO).—To kynurenine (1·2 g.) in formic acid (4 ml.) was added acetic anhydride (1·5 ml.). Crystals soon separated, and after 2 hours a little water was added, to give the N′-*acetyl*-N^{α}-*formyl* compound in almost theoretical yield, as cream-coloured crystals from ethanol, decomposing at 224° on fairly rapid heating (Found: C, 55·8, 55·9; H, 5·0, 5·1; N, 9·9, 10·1. $C_{13}H_{14}O_5N_2$ requires C, 56·1; H, 5·0; N, 10·1%).

N′N^{α}-*Diacetyl*-DL-*kynurenine* (IV; R = R′ = Ac).—To N^{α}-acetylkynurenine (0·8 g.; see below) in formic acid (2 ml.) was added acetic anhydride (0·32 ml.). Crystals soon separated, and after 2 hours addition of a little water completed precipitation. The diacetyl compound formed cream-coloured crystals from ethanol, decomposing at 185° (Found: C, 57·3, 57·6; H, 5·7, 5·9; N, 9·8. Calc. for $C_{14}H_{16}O_5N_2$: C, 57·5; H, 5·5; N, 9·6%). The ultra-violet absorption spectrum determined in 20% alcohol showed maxima at 260 (ε = 9760) and 320 mμ (ε = 2980). Diacetylkynurenine has been made by Butenandt *et al.* (*Z. physiol. Chem.*, 1943, **279**, 27) by treatment of kynurenine with keten.

Formylation of Phenacylglycine, o-Nitrophenacylglycine, and Tryptophan.—To the amino-acid dissolved in formic acid was added one equivalent of acetic anhydride. After 2 hours separation of the product, obtained in all cases in high yield, was completed by the addition of water. *Formyl*-DL-*phenacylglycine*, colourless prisms (from methanol), decomposed at 199° (Found: C, 59·9; H, 5·3; N, 6·6. $C_{11}H_{11}O_4N$ requires C, 59·7; H, 5·0; N, 6·3%). *Formyl*-DL-*o*-*nitrophenacylglycine* (VI; R = CHO), silky white needles from methanol, decomposed at 178° (Found: C 49·9; H, 3·9; N, 10·7. $C_{11}H_{10}O_6N_2$ requires C, 49·6; H, 3·8; N, 10·5%). *Formyl*-DL-*tryptophan*, colourless crystals (from water), had m. p. 167—168° (slight decomp.) (Found: C, 61·7; H, 5·2. $C_{12}H_{12}O_3N_2$ requires C, 62·1; H, 5·2%).

N'-Formyl-DL-*kynurenine* (II).—A cooled mixture of acetic anhydride (1 ml.) in formic acid (2 ml.) was set aside for 30 minutes and then added to kynurenine (2·08 g.) in formic acid (4·5 ml.). After 2 hours the mixture was poured, with shaking, into ether, and after a few hours at 0° the product was isolated (2·55 g., almost 100%). The product contained approx. 0·3 mol. of formic acid, as determined on a sample by electrometric titration. The material was taken up in hot water, the calculated amount of N-sodium hydroxide added, and the whole cooled with shaking. Cream-coloured needles separated which were obviously highly hydrated, as judged by their shrinkage on drying. The material when recrystallised from water and dried in a vacuum-desiccator over phosphoric oxide overnight still contained some water, which was removed in a vacuum at an elevated temperature. The dried compound decomposed at 162° (Found : C, 55·8; H, 5·5; N, 12·1. $C_{11}H_{12}O_4N_2$ requires C, 55·9; H, 5·1; N, 11·9%). The ultra-violet absorption spectrum determined in M/5-phosphate buffer, pH 7·0, diluted 1 : 10, showed maxima at 260 (ε = 10,980) and 321 mμ (ε = 3750).

$N'N^{\alpha}$-*Diformyl*-DL-*kynurenine* (IV; R = R' = CHO) was obtained on further formylation of *N'*-formylkynurenine. It formed cream-coloured crystals (from ethanol), decomposing at 213° (Found : C, 54·6; H, 4·7; N, 10·4. $C_{12}H_{12}O_5N_2$ requires C, 54·6; H, 4·5; N, 10·6%).

N-*Acetyl*-DL-*o*-*nitrophenacylglycine* (VI; R = Ac).—*o*-Nitrophenacylglycine hydrochloride (5·5 g.) in N-sodium hydroxide (80 ml.) was shaken with acetic anhydride (4 ml.). When the reaction was complete, dark material was removed from the alkaline solution by extraction with ethyl acetate. The aqueous fraction was acidified and the product allowed to separate at 0°. Recrystallisation from ethyl acetate gave the *acetyl* compound as cream-coloured crystals, m. p. 190° (Found : C, 51·2; H, 3·9; N, 9·9. $C_{12}H_{12}O_6N_2$ requires C, 51·4; H, 4·3; N, 10·0%).

N^{α}-*Acetyl*-DL-*kynurenine* (III).—The nitro-compound in ethanol solution was hydrogenated over palladium–charcoal at room temperature and atmospheric pressure, the theoretical amount of hydrogen being taken up smoothly. After removal of the catalyst and solvent the *product* was obtained as a rather hygroscopic yellow glass which could not be induced to crystallise (Found : C, 57·7; H, 5·5; N, 11·0. $C_{12}H_{14}O_2N_2$ requires C, 57·6; H, 5·6; N, 11·2%). It was shown to be homogeneous by paper-chromatography (cf. Dalgliesh, Knox, and Neuberger, *loc. cit.*) and on paper gave an immediate orange Ehrlich's reaction, no ninhydrin reaction, could be diazotised and coupled, and showed the same colour of fluorescence as kynurenine.

N^{α}-*Acetyl*-*N'*-*formyl*-DL-*kynurenine* (IV; R = CHO, R' = Ac).—To a mixture of formic acid (2 ml.) and acetic anhydride (0·6 ml.) was added after 30 minutes a solution of N^{α}-acetylkynurenine (1·7 g.) in formic acid (4 ml.). After 2 hours separation of the product in high yield was completed by addition of water and setting the whole aside in the cold room. The N^{α}-*acetyl*-*N'*-*formyl* compound formed cream-coloured crystals (from ethanol), decomposing at 194° (Found : C, 55·9; H, 4·9. $C_{13}H_{14}O_5N_2$ requires C, 56·1; H, 5·0%).

N^{α}-*Carbethoxy*-DL-*o*-*nitrophenacylglycine* (VI; R = CO_2Et).—Carbethoxylation carried out under Schotten–Baumann conditions as for acetylation above, but using ethyl chloroformate, gave the *carbethoxy*-compound, stout almost colourless needles (from ethyl acetate–light petroleum), m. p. 154° (Found : C, 50·6; H, 4·6; N, 8·8. $C_{13}H_{14}O_7N_2$ requires C, 50·3; H, 4·5; H, 9·0%).

N^{α}-*Carbethoxy*-DL-*kynurenine*.—Hydrogenation as for the N^{α}-acetyl compound gave a gum which was induced to crystallise after some difficulty, recrystallisation from ethyl acetate–light petroleum giving pale yellow crystals of N^{α}-*carbethoxy*-DL-*kynurenine*, m. p. 120° (Found : N, 10·1. $C_{13}H_{16}O_5N_2$ requires N, 10·0%). The material behaved like kynurenine in the colour of its fluorescence and in its reaction to diazotisation and Ehrlich's reagent, but did not give a ninhydrin reaction.

Formylation of Carbethoxykynurenine.—Carbethoxykynurenine (0·2 g.) in ethyl formate (3 ml.) was heated in a sealed tube at 100° for 14 hours. Removal of excess of ethyl formate left a glass which appeared probably to be N^{α}-*carbethoxy*-*N'*-*formyl*-DL-*kynurenine* (Found : N, 8·8. $C_{14}H_{16}O_6N_2$ requires N, 9·1%), but its purification and hydrolysis were not investigated.

I thank Dr. A. Neuberger, F.R.S., for his valuable interest and advice, Dr. W. E. Knox for enzyme tests, and Mr. A. J. Tilley for technical assistance.

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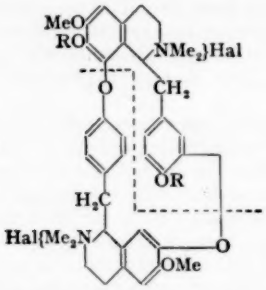
30. *Synthetic Neuromuscular Blocking Agents. Part II.* Bis(quaternary Ammonium Salts) derived from Laudanosine.*

By E. P. Taylor.

In continuation of earlier work, some heterocyclic bis(quaternary ammonium salts) have been prepared which show close chemical similarities to (+)-*OO*-dimethyltubocurarine iodide and possess greater curarising activity in the rabbit than does (+)-tubocurarine chloride.

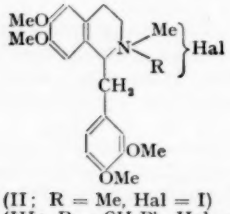
In the search for new curarising agents, the resemblance between the structure of (+)-tubocurarine chloride (I) and those of certain other alkaloids has been taken into consideration by various workers. Thus, Craig and Tarbell (*J. Amer. Chem. Soc.*, 1948, **70**, 2783) prepared and investigated the pharmacological properties of, *inter alia*, *N*-methyllaudanosinium iodide (II) and *N*-benzyl-laudanosinium bromide (III), both of which closely resemble one half of the molecule of (+)-*OO*-dimethyltubocurarine iodide (IV).



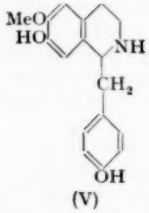
(I; R = H, Hal = Cl)

(IV; R = Me, Hal = I)

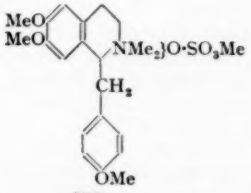
As might be expected, both these salts possess some, although very slight, curare-like activity in mice. More recently, Finkelstein (*ibid.*, 1951, **73**, 550) synthesised the alkaloid coclaurine (V), and its fully methylated quaternary salt (VI), but both were devoid of curare-like activity in dogs at the dosage level used.



(II; R = Me, Hal = I)
(III; R = CH_2Ph, Hal = Br)



(V)



(VI)

In Part I of this series (*J.*, 1951, 1150) it was shown that certain heterocyclic decamethylenebis(quaternary ammonium salts) possess strong neuromuscular blocking activity of true curare type. Consequently, it was decided to prepare some bisquaternary salts of laudanosine. In addition to polymethylene derivatives (VII—XI), compounds (XII and XIII) containing the $[CH_2]_4{\cdot}O{\cdot}[CH_2]_4$ and the $[CH_2]_5{\cdot}O{\cdot}[CH_2]_5$ chain were prepared. Although the products were recrystallised to analytical purity, to remove any monoquaternary salt that might be formed, no attempt was made to ensure complete separation of the diastereoisomers.

Dr. H. O. J. Collier found these bisquaternary laudanosinium salts to be powerful neuromuscular blocking agents, in animals, of true curare type, being antagonised by neostigmine and causing the typical flaccid paralysis in the chick. The paralysing

* Part I, *J.*, 1951, 1150.

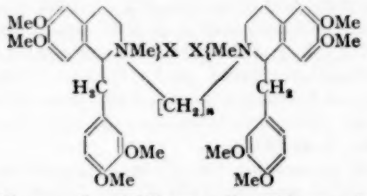
activities of these substances in rabbits and mice are indicated in the table, the last two substances being inserted for comparison. A brief note on these compounds has already appeared (Taylor and Collier, *Nature*, 1951, **167**, 692) and the full pharmacological results will be reported elsewhere (Collier and Macauley, in the press).

Paralysing activity of bislaudanosinium salts.

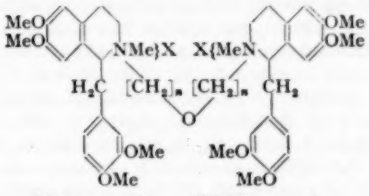
(μg. per kg. of body weight.)

Compound	Chain	Approx. M.E.D. (rabbit)	E.D.50 (mouse)	Compound	Chain	Approx. M.E.D. (rabbit)	E.D.50 (mouse)
(VII)	Octamethylene	47	260	(XII)	$[CH_2]_4{\cdot}O{\cdot}[CH_2]_4$	70	—
(VIII)	Nonamethylene	26	160	(XIII)	$[CH_2]_5{\cdot}O{\cdot}[CH_2]_5$	200	—
(IX)	Decamethylene	31	300	(I)	(+)-Tubocurarine chloride	100	70
(X)	Undecamethylene	55	410				
(XI)	Dodecamethylene	90	760	(IV)	(+)-*OO*-Dimethyltubocurarine iodide	10	120

Although, in the tubocurarine molecule, the two quaternary groups are separated by a chain containing an ether linkage, the two compounds (XII and XIII) with an ether linkage are weaker than the corresponding polymethylene derivatives (VIII) and (X). In rabbits, the two most active compounds are the nonamethylene (VIII) and the decamethylene (IX) derivative. Although (VIII) shows considerably greater paralysing



(VII, $n = 8$) (VIII; $n = 9$) (IX; $n = 10$) (X; $n = 11$) (XI; $n = 12$)



(XII; $n = 4$) (XIII; $n = 5$)

activity in mice than does (IX), it appears to liberate more histamine in man. Moreover, it will be observed that (+)-*OO*-dimethyltubocurarine iodide (IV), although weaker in mice than is (+)-tubocurarine chloride (I), is a much more powerful paralysant in rabbits (and also in man). For these and other reasons (Bodman, in the press; Collier and Macauley, *loc. cit.*), the decamethylene derivative, under the name "Laudolissin" or "Compound 20" was selected for further investigation. Its action in human volunteers has been studied by Dr. Richard Bodman of the University of Bristol. Preliminary results indicate that it possesses approximately half to two-thirds of the curarising activity of (+)-tubocurarine chloride in man, that it is antagonised by neostigmine, and that it appears not to produce undesirable side-effects. This compound is being subjected to extensive clinical trials.

The bisquaternary salts were prepared by the two general methods described in Part I (*loc. cit.*), by interaction of the appropriate dihalide with tetrahydropapaverine or laudanosine, followed in the former case by treatment of the resultant bistertiary amine with an alkyl halide or sulphate. The five polymethylene di-iodides required have already been described; however, a new and very simple general method of preparing these compounds in excellent yield is to reflux a solution of the appropriate glycol in hydriodic acid (*d* 1·94); the iodide separates as a heavy oil, which can be easily extracted and purified. 4 : 4′-Di-iododibutyl ether and 5 : 5′-di-iododiamyl ether, which appear to be new, were obtained by treatment of the known dichloro-compounds with sodium iodide in acetone. There are various catalytic methods for the reduction of papaverine (Craig and Tarbell, *loc. cit.*) and of its methiodide (Barltrop and Taylor, *J.*, 1951, 108), but, in the apparatus available, these were unsuitable for the preparation of tetrahydropapaverine or of laudanosine in the quantities required. Tetrahydropapaverine was obtained in 60% yield by reduction of papaverine with zinc and hydrochloric acid (cf. Pyman, *J.*, 1909, 1614,

who obtained a 39% yield by reduction with tin and aqueous-alcoholic hydrochloric acid). A similar reduction of papaverine methiodide yielded laudanosine in 73% yield (67% overall yield based on papaverine) (Pyman and Reynolds, *J.*, 1910, 1323, obtained an overall yield of 78%).

Experimental

(Analyses are by Drs. Weiler and Strauss, Oxford. M. p.s and b. p.s are uncorrected.)

Preparation of Polymethylene Di-iodides.—A mixture of decamethylene glycol (140 g.) and hydriodic acid (618 g.; *d* 1·94; 100% excess) was mechanically stirred and heated under reflux for 6 hours. After cooling and dilution with water the precipitated heavy oil was extracted with ether, the extract washed successively with water, sodium hydrogen carbonate solution, sodium thiosulphate solution, and water, and dried (Na_2SO_4), the ether recovered, and the residue distilled *in vacuo*. The fraction of b. p. 197—200°/12 mm. (299 g.), which solidified, was recrystallised from alcohol, giving decamethylene di-iodide (281 g., 88·5%), m. p. 28—29°.

In a similar manner, octamethylene (80%), nonamethylene (83%), undecamethylene (81%), and dodecamethylene (78·5%) di-iodides were obtained from the corresponding glycols.

4 : 4′-*Di-iododibutyl Ether*.—4 : 4-Dichlorodibutyl ether (Alexander and Schniepp, *J. Amer. Chem. Soc.*, 1948, **70**, 1840; Alexander and Towles, *Org. Synth.*, 1950, **30**, 27) (15 g.) was added to a solution of sodium iodide (33·9 g., 50% excess) in dry acetone (300 ml.), and the mixture refluxed for 7 days. The precipitated salt was then filtered off and washed with acetone, the solvent recovered from the combined filtrate and washings, and the residue dissolved in ether, washed (water, sodium thiosulphate solution, and water), and dried (Na_2SO_4). After recovery of the solvent, the residue was distilled *in vacuo*, and the fraction of b. p. 100—105°/0·02 mm. redistilled, giving **4 : 4′-*di-iododibutyl ether*** (23 g., 80%), b. p. 102—104°/0·05 mm. (with slight decomp.) (Found : C, 26·0; H, 4·4; I, 65·6. $C_8H_{16}OI_2$ requires C, 25·1; H, 4·2; I, 66·5%).

Similarly, **5 : 5′**-dichlorodiamyl ether (Alexander and Schniepp, *loc. cit.*, 1948) was converted into **5 : 5′-*di-iododiamyl ether*,** b. p. 136—138°/0·1 mm. (with slight decomp.) (Found : C, 29·6; H, 5·3; I, 62·8. $C_{10}H_{20}OI_2$ requires C, 29·3; H, 4·9; I, 61·95%).

Tetrahydropapaverine Hydriodide.—A solution of papaverine (50 g.) in hydrochloric acid (500 ml.) and water (3750 ml.) was gently refluxed, whilst zinc dust (150 g.) was added in small portions during 2 hours. After a further 24 hours' refluxing, fresh quantities of hydrochloric acid (500 ml.) and zinc dust (150 g.) were added, and refluxing was continued for a total time of 54 hours. The hot solution was then filtered from excess of zinc, the latter was washed with hot water, the combined filtrate and washings were concentrated *in vacuo* to half-volume, then made alkaline with excess of ammonia and cooled. The precipitated bases were extracted with ether (there was a small quantity of insoluble material), the ethereal extract was dried (Na_2SO_4), and the solvent recovered. The oily residue was dissolved in hot dilute hydrochloric acid, and excess of powdered potassium iodide (30 g.) added slowly with stirring. After cooling, the amorphous precipitate was filtered off, washed with cold water, sucked dry, and boiled with alcohol (500 ml.), whereupon it crystallised. After cooling, filtration, and drying, crude tetrahydropapaverine hydriodide (49 g.; m. p. 242—244°) was obtained. This was recrystallised from water, giving pure material (42 g.), m. p. 257—258° [Pyman, *J.*, 1909, 1614, gives m. p. 259—260° (corr.)].

Preparation of Bis(tertiary Amines).—Dihydrochloride of **1 : 10-*bis*-[1-(3 : 4-*dimethoxybenzyl*)-1 : 2 : 3 : 4-*tetrahydro*-6 : 7-*dimethoxy*isoquinolino]*decane*.** A mixture of decamethylene di-iodide (11·8 g.), tetrahydropapaverine hydriodide (29·7 g., 2·1 mols.), anhydrous potassium carbonate (15 g.), and 95% alcohol (360 ml.) was refluxed for 104 hours. The alcohol was then distilled off, and any residual water removed by azeotropic distillation with benzene. The residue was dissolved as far as possible in hot dry benzene and filtered, and the solvent recovered, finally *in vacuo*. The oily residue was dissolved in the minimum quantity of anhydrous alcohol, and acidified to Congo-red with dry alcoholic hydrogen chloride. After cooling, the resultant dihydrochloride was filtered off, washed with cold alcohol, and dried, giving crude material of m. p. 236—238° (25·6 g., 95%). When this was recrystallised from boiling water (500 ml.), the *product* (20·5 g., 76%) was obtained as a colourless powder, m. p. 242—244° (decomp.) (Found : C, 66·7; H, 7·75; N, 3·2; Cl, 7·95. $C_{50}H_{70}O_8N_2Cl_2$ requires C, 66·9; H, 7·9; N, 3·1; Cl, 7·9%).

The following analogues were prepared in a similar manner :

1 : 8-*Bis*-[1-(3 : 4-*dimethoxybenzyl*)-1 : 2 : 3 : 4-*tetrahydro*-6 : 7-*dimethoxy*isoquinolino]*octane dihydrochloride* (from water), m. p. 256—258° (decomp.) (Found : N, 3·0; Cl, 8·5. $C_{48}H_{66}O_8N_2Cl_2$

requires N, 3·2; Cl, 8·2%); 1 : 11-*bis*-[1-(3 : 4-*dimethoxybenzyl*)-1 : 2 : 3 : 4-*tetrahydro*-6 : 7-*dimethoxy*isoquinolino]*undecane dihydrochloride* (from alcohol–ether), softens at 207°, m. p. 214—215° (Found : N, 3·05; Cl, 7·7. $C_{51}H_{72}O_8N_2Cl_2$ requires N, 3·1; Cl, 7·8%); 1 : 12-*bis*-[1-(3 : 4-*dimethoxybenzyl*-1 : 2 : 3 : 4-*tetrahydro*-6 : 7-*dimethoxy*isoquinolino]*dodecane dihydrochloride* (from alcohol–ether), m. p. 224—226° (decomp.) (Found : N, 3·0; Cl, 8·0. $C_{52}H_{74}O_8N_2Cl_2$ requires N, 3·0; Cl, 7·7%); and *bis*-{5-[1-(3 : 4-*dimethoxybenzyl*)-1 : 2 : 3 : 4-*tetrahydro*-6 : 7-*dimethoxy*-isoquinolino]*amyl*} *ether dihydrochloride* (from alcohol–ether), softens at 198—200°, m. p. 208—210° (Found : N, 3·2; Cl, 7·9. $C_{50}H_{70}O_9N_2Cl_2$ requires N, 3·1; Cl, 7·8%).

Preparation of Bis(quaternary Ammonium Salts).—Note : the analyses of all quaternary compounds described below were carried out on material dried *in vacuo* at 100°.

Method I. *Decamethylenebis*-[1-(3 : 4-*dimethoxybenzyl*)-1 : 2 : 3 : 4-*tetrahydro*-6 : 7-*dimethoxy*-2-*methyl*isoquinolinium *methosulphate*] (IX). The dihydrochloride (20 g.) of 1 : 10-bis-[1-(3 : 4-dimethoxybenzyl)-1 : 2 : 3 : 4-tetrahydro-6 : 7-dimethoxy*iso*quinolino]decane was dissolved in boiling water (700 ml.), made alkaline with sodium hydroxide, and saturated with sodium chloride, and the brown oil which separated extracted with hot benzene. The benzene solution was shaken with solid potassium hydroxide, filtered, and concentrated to approx. 100 ml. A solution of methyl sulphate (8·45 g., 50% excess) in dry benzene (25 ml.) was added, and the mixture refluxed for 48 hours. A heavy oil soon separated, which slowly thickened to a gummy solid. The benzene was then decanted off, and the residue washed three times by decantation with hot benzene and then dried *in vacuo*, yielding a light brown friable solid. This was dissolved in hot anhydrous alcohol, ether was added to the warm solution, and the mixture left to crystallise slowly. After two further recrystallisations, the *product* was obtained as a cream-coloured granular powder (10 g.), m. p. 172—174° after darkening at 164—166° (Found : C, 60·1; H, 7·75; N, 2·5; S, 5·9. $C_{54}H_{80}O_{16}N_2S_2$ requires C, 60·2; H, 7·5; N, 2·6; S, 5·95%).

The *undecamethylene* (X) (from alcohol–ether), m. p. 187—189° after darkening at 170° (Found : C, 60·8; H, 7·3; N, 2·7; S, 5·85. $C_{55}H_{82}O_{16}N_2S_2$ requires C, 60·55; H, 7·6; N, 2·6; S, 5·9%), and the *dodecamethylene* analogue (XI) (from alcohol–ether), softens at 202°, m. p. 205—207° (Found : C, 61·0; H, 7·65; N, 2·7; S, 5·9. $C_{56}H_{84}O_{16}N_2S_2$ requires C, 60·9; H, 7·7; N, 2·5; S, 5·8%), were similarly prepared, as was the 6-*oxaundecamethylene* analogue (XIII) (from alcohol–ether), darkens at 142°, m. p. 154—156° (Found : C, 58·8; H, 7·4; N, 2·6; S, 5·9. $C_{54}H_{80}O_{17}N_2S_2$ requires C, 59·3; H, 7·4; N, 2·6; S, 5·9%).

Method II. *Decamethylenebis*-[1-(3 : 4-*dimethoxybenzyl*)-1 : 2 : 3 : 4-*tetrahydro*-6 : 7-*dimethoxy*-2-*methyl*isoquinolinium *iodide*] (IX). Decamethylene di-iodide (1 g.) and laudanosine (3·63 g., 100% excess) in dry benzene (25 ml.) were refluxed for 108 hours. The solution became faintly cloudy after 4 hours, and slowly deposited a heavy oil, which solidified on cooling. After filtration the residue was crushed, thoroughly washed with benzene, and dried. The pale yellow friable residue was dissolved in methyl alcohol, and poured into excess of ether with stirring. After repetition of this precipitation, it was possible to recrystallise the material from methyl alcohol–ether in the normal manner. The *product* (1·1 g.) was obtained as cream-coloured granules, which softened at 136—138° and melted at 146—148° (Found : C, 55·9; H, 6·65; N, 2·4; I, 22·6. $C_{52}H_{74}O_8N_2I_2$ requires C, 56·3; H, 6·7; N, 2·5; I, 22·9%).

The corresponding *bromide*, prepared from decamethylene dibromide, was obtained as granules (from butyl alcohol–ether), which softened at 93—95° and melted at 105—107° (Found : N, 2·65; Br, 16·0. $C_{52}H_{74}O_8N_2Br_2$ requires N, 2·8; Br, 15·8%).

Iodides were prepared in a similar manner of the *octamethylene* (VII), granules (from butyl alcohol–ether), softens at 127—129°, m. p. 135—137° (Found : N, 2·55; I, 23·3. $C_{50}H_{70}O_8N_2I_2$ requires N, 2·6; I, 23·5%), *nonamethylene* (VIII), granules (from butyl alcohol), softens at 130—132°, m. p. 139—141° (Found : N, 2·7; I, 23·1. $C_{51}H_{72}O_8N_2I_2$ requires N, 2·6; I, 23·2%), and 5-*oxanonamethylene* analogue (XII) granules (from butyl alcohol), softens slightly at 118—120°, m. p. 130—132° (Found : N, 2·3; I, 22·8. $C_{50}H_{70}O_9N_2I_2$ requires N, 2·55; I, 23·2%).

I thank Mr. W. C. Austin for technical assistance, Dr. H. O. J. Collier for supplying the pharmacological results, Dr. Richard Bodman for supplying preliminary results in human volunteers, and the Directors of Messrs. Allen and Hanburys Ltd. for permission to publish this work.

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31. *Some Aliphatic Thiols and Their Derivatives. Part II.* Thiols Derived from 2 : 2′-Dichlorodiethyl Sulphide and its Analogues. Nickel Complexes from 1 : 5-Dithiols.*

By John Harley-Mason.

Reaction of 2 : 2′-dichlorodiethyl sulphide with thiourea, followed by treatment with alkali, gives 2 : 2′-dimercaptodiethyl sulphide which forms a highly crystalline deep red nickel complex; similar complexes are obtained with di-(2-mercaptoethyl)methylamine and 2 : 2′-dimercaptotriethylamine. The structure of these remarkable complexes is discussed.

A dithiol is similarly obtained from 1 : 2-di-(2-chloroethylthio)ethane, but did not give satisfactory complexes.

In the course of work during 1943—1944 on the development of reagents for the detection and identification of war gases, the reaction of thiourea with 2 : 2′-dichlorodiethyl sulphide (mustard gas) was examined: the bisthiuronium chloride obtained was found to give a deep red precipitate (soluble in chloroform) when treated with alkaline nickel solutions. Since the reaction was sensitive and specific further investigations were made.

Thiuronium salts are readily decomposed by alkali (Arndt, *Ber.*, 1921, **54**, 2236), giving mercaptans, so that it seemed probable that the red material was a nickel complex of 2 : 2′-dimercaptodiethyl sulphide (I), first obtained as one of the products of the action

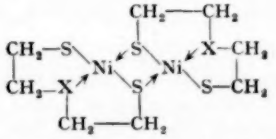
$S(CH_2{\cdot}CH_2{\cdot}SH)_2$ (I) $\quad MeN(CH_2{\cdot}CH_2{\cdot}SH)_2$ (II) $\quad EtN(CH_2{\cdot}CH_2{\cdot}SH)_2$ (III)

of alkali on ethane-1 : 2-bisthiuronium bromide by Matthias (*Bols. Fac. Fil. cienc. Letras, Univ. Sao Paulo*, 1942, **14**, Quim. No. 1, 75; *Chem. Abs.*, 1946, **40**, 2792). In the present work, (I) was prepared in high yield by the reaction of mustard gas with two mols. of thiourea in boiling ethanol and decomposition of the resulting bisthiuronium chloride with sodium hydroxide. Lead and mercury derivatives of (I) were prepared by Matthias (*loc. cit.*), and similar precipitates insoluble in water and organic solvents were obtained from (I) and salts of copper, cadmium, cobalt, palladium, platinum, and gold. On addition of (I), with shaking, to an ammoniacal nickel sulphate solution, the deep red precipitate, which formed at once, slowly crystallised and, in contradistinction to other metal derivatives, was readily soluble in halogenated solvents such as chloroform, trichloroethylene, ethylene dibromide, and bromoform, giving deep red solutions. Recrystallisation from chloroform gave the complex (IV; X = S) as large garnet-red prisms, insoluble in water and stable to alkali but decomposed by mineral acids and by potassium cyanide solution with regeneration of the dithiol. Very similar complexes (IV; X = MeN and X = EtN) were obtained in the same way from the structurally similar dithiols, di-(2-mercaptoethyl)methylamine (II) and 2 : 2′-dimercaptotriethylamine (III) (for preparation, see Part I, *loc. cit.*). Elementary analysis showed that in all three cases the complexes contained one atom of nickel per molecule of dithiol, but molecular-weight determinations showed that this empirical formula must be doubled. 2 : 2′-Dimercaptodiethyl ether (Backer and Stienstra, *Rec. Trav. chim.*, 1933, **52**, 1037) and 2 : 2′-dimercaptodiethyl sulphoxide (V) (see below), in which it would be expected that the central hetero-atom would be incapable of co-ordination, were found to give entirely different, insoluble, yellowish-brown nickel derivatives, so that in the red complexes (IV) the central sulphur or nitrogen atom must clearly be co-ordinated with the metal. The red colour and solubility in organic solvents suggest that these are non-ionic "square complexes" of 4-covalent nickel (cf. nickel dimethylglyoxime) and on the basis of the above facts the bridged structure (IV) is assigned to them.

An interesting possibility of stereoisomerism then arises. In the case of (IV; X = S), the two nickel and the six sulphur atoms must clearly be in one plane, but, owing to the pyrimidal configuration of 3-covalent sulphur, two possibilities arise: (*a*) the two pairs

* Part I, *J.*, 1947, 320.

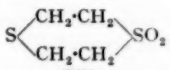
of ethylene bridges are above the plane, giving a *cis*-form; or (*b*) one pair is above the plane and the other below, giving a *trans*-form. However, repeated fractional crystallisation of the material gave no indication of inhomogeneity, and it is probable that only one form is obtained.



(IV)

Formation of the complex may be used as the basis of a sensitive and specific test for mustard gas and the " nitrogen mustards " corresponding to the dithiols (II) and (III). Contaminated air is aspirated through a 5% solution of thiourea in 2-ethoxyethanol, or alternatively a contaminated article is washed with such a solution. The solution is boiled for two minutes and then poured into an ammoniacal nickel sulphate solution and shaken with chloroform. The appearance of a red colour in the chloroform layer constitutes a positive test. On a test-tube scale, 0·04 mg. of the war gas can be detected visually. All other war gases tested gave negative results.

2 : 2′-Dichlorodiethyl sulphoxide reacted with thiourea less readily than did the sulphide, giving a bisthiuronium chloride which on treatment with alkali gave only white polymeric material. When, however, the decomposition was carried out in the presence of ammoniacal nickel sulphate a nickel derivative was precipitated, forming yellowish plates, insoluble in water and organic solvents. Analysis indicated that it was formed from one molecule of the sulphoxide (V) and one atom of nickel. Decomposition of the

(V) $OS(CH_2{\cdot}CH_2{\cdot}SH)_2$  (VI) $(CH_2{\cdot}S{\cdot}CH_2{\cdot}CH_2{\cdot}SH)_2$ (VII)

nickel derivative with potassium cyanide gave first an oil, giving a strong thiol reaction with sodium nitroprusside, but the oil was rapidly converted into a sparingly soluble amorphous white solid which gave no nitroprusside reaction. It appears therefore that the dithiol (V) is too unstable to be characterised and that the sulphoxide group oxidises the thiol groups with the formation of polymer.

2 : 2′-Dichlorodiethyl sulphone reacted with difficulty with thiourea: however, on long heating of the reactants in 2-ethoxyethanol a bisthiuronium chloride was obtained. Decomposition with alkali again gave mainly white amorphous polymer, but a small amount of 1 : 4-dithian 1 : 1-dioxide (VI) was isolated, presumably arising from intramolecular elimination of hydrogen sulphide from the dithiol at first formed.

1 : 2-Di-(2-chloroethylthio)ethane readily gave a bisthiuronium chloride which with alkali gave 1 : 2-di-(2-mercaptoethylthio)ethane (VII) as a low-melting solid. It was hoped that this dithiol, with two central atoms available for co-ordination, would act as a quadridentate reagent for metals capable of forming " square complexes." Attempts to prepare such complexes were however unsuccessful. Similarly, 2 : 2′ : 2″-trimercaptotriethylamine (Part I, *loc. cit.*), which might be expected to function as a quadridentate reagent for tetrahedral 4-covalent metals, also failed to give satisfactory complexes. It is of interest that 2 : 2′-dimercaptodiethylamine, despite its close relation to (II) and (III), failed to give a complex of type (IV) with nickel. This may possibly be due to a difference in the co-ordinating ability of secondary and tertiary nitrogen.

The action of chlorine on aqueous solutions of *iso*thioureas has been shown by Johnson and Sprague (*J. Amer. Chem. Soc.*, 1936, **58**, 1348) to give sulphonyl chlorides. Treatment of the bisthiuronium chloride from 2 : 2′-dichlorodiethyl sulphide with chlorine at 0° gave a solid bis-sulphonyl chloride, characterised as the diamide and dianilide. The same product was obtained from the corresponding sulphoxide and sulphone, so that evidently oxidation of the central sulphur atom occurred in the first two cases, the product being in all cases (diethyl sulphone)-2 : 2′-bis-sulphonyl chloride.

Experimental

2 : 2′-*Dimercaptodiethyl Sulphide.*—Thiourea (50 g.), redistilled mustard gas (50 g.), and ethanol (150 c.c.) were refluxed for 2 hours. After being kept overnight the product was filtered off and washed with water. (*Diethyl sulphide*)-2 : 2′-*bisthiuronium chloride* formed small prisms, m. p. 116—118° (decomp.) after softening at 108° (Found : N, 18·2. $C_6H_{16}N_4S_2Cl_2$ requires N, 18·0%).

To a solution of the thiuronium salt (30 g.) in water (100 c.c.) a solution of sodium hydroxide (8 g.) in water (20 c.c.) was added. The mixture was heated for 10 minutes on a boiling water-bath, then cooled, and the oily dithiol taken up in ether. After drying (Na_2SO_4) and removal of the solvent, 2 : 2′-dimercaptodiethyl sulphide distilled at 135—137°/18 mm. (11·5 g., 70%) (Found : C, 30·9; H, 6·7. Calc. for $C_4H_{10}S_3$: C, 31·2; H, 6·5%).

Formation of Nickel Complexes.—The dithiol (I) (5 g.) was added dropwise, with vigorous shaking, to a solution of nickel sulphate (12 g.) in water (150 c.c.) and ammonia (*d* 0·88; 25 c.c.). The red precipitate became crystalline when kept and was then collected and recrystallised from chloroform. The *nickel* complex (IV; X = S) formed garnet-red prisms which decomposed without melting when heated [Found : C, 22·9; H, 4·0; Ni, 27·8%; *M* (cryoscopic in ethylene dibromide), 441. $C_6H_{16}S_6Ni_2$ requires C, 22·7; H, 3·8; Ni, 27·9%; *M*, 422].

In the same manner the *nickel* complex (IV; X = MeN) was prepared from di-(2-mercaptodiethyl)methylamine (II) (Found : C, 29·0; H, 5·3; Ni, 28·7. $C_{10}H_{22}N_2S_4Ni_2$ requires C, 28·8; H, 5·3; Ni, 28·4%), and the *nickel* complex (IV; X = EtN) from 2 : 2′-dimercaptotrimethylamine (III) [Found : C, 32·0; H, 6·1; Ni, 27·0%; *M* (cryoscopic in bromoform), 495. $C_{12}H_{26}N_2S_4Ni_2$ requires C, 32·4; H, 5·85; Ni, 26·6%; *M*, 444]. The *nickel* complex, which formed an amorphous light brown powder insoluble in water and organic solvents, was similarly prepared from 2 : 2′-dimercaptodiethyl ether (Found : Ni, 30·1. $C_4H_8OS_2Ni$ requires Ni, 30·2%)

Reaction of 2 : 2′-*Dichlorodiethyl Sulphoxide with Thiourea.*—2 : 2′-Dichlorodiethyl sulphoxide (36 g.) and thiourea (31 g.) were refluxed in ethanol (120 c.c.) for 8 hours. Next morning the product which had crystallised was collected and washed with acetone. (*Diethyl sulphoxide*)-2 : 2′-*bisthiuronium chloride* formed small white plates, m. p. 198° (decomp.) (Found : N, 17·0. $C_6H_{16}ON_4S_2Cl_2$ requires N, 17·1%). A solution of the thiuronium salt (19·6 g.) in water (100 c.c.) was added to a solution of nickel sulphate (16 g.) in water (100 c.c.) and aqueous ammonia (*d* 0·88; 50 c.c.). The mixture was heated on a boiling water-bath, whereupon the product rapidly separated. The material was filtered off hot and washed with water and alcohol. The *nickel* complex of 2 : 2′-dimercaptodiethyl sulphoxide formed bronze-yellow plates, insoluble in water and organic solvents (Found : Ni, 25·8. $C_4H_8OS_2Ni$ requires Ni, 26·5%). The complex (5 g.) was added with stirring to a solution of potassium cyanide (7 g.) in water (30 c.c.), the solid rapidly dissolving with evolution of heat. After filtration from traces of undissolved material the solution was acidified with dilute hydrochloric acid and extracted with peroxide-free ether. After being dried (Na_2SO_4), the solvent was removed, leaving an oil with a " mercaptan " odour and giving an intense nitroprusside reaction. The oil rapidly changed spontaneously into a white amorphous polymeric solid which no longer gave a nitroprusside reaction.

Reaction of 2 : 2′-*Dichlorodiethyl Sulphone with Thiourea.*—2 : 2′-Dichlorodiethyl sulphone (38 g.) and thiourea (31 g.) were heated in 2-ethoxyethanol (100 c.c.) for 8 hours on a boiling water-bath. When the cooled mixture was set aside for 2 days the (*diethyl sulphone*)-2 : 2′-*bisthiuronium chloride*, m. p. 211°, which had separated as small white prisms was collected and washed with acetone (Found : N, 16·2. $C_6H_{16}O_2N_4S_2Cl_2$ requires N, 16·3%).

The thiuronium salt (14 g.) was dissolved in water (100 c.c.) and a solution of sodium hydroxide (3·2 g.) in water (10 c.c.) was added. The *iso*thiourea was at once precipitated as small white plates. The mixture was heated at 100° for 0·5 hour, whereupon the crystalline precipitate slowly changed into white amorphous solid. This was filtered off hot and the filtrate on cooling slowly deposited small colourless prisms of 1 : 4-dithian 1 : 1-dioxide, m. p. 206° after recrystallisation from water (Found : C, 31·5; H, 5·5. Calc. for $C_4H_8O_2S_2$: C, 31·6; H, 5·3%). Alexander and McCombie (*J.*, 1931, 1913) give m. p. 206°.

1 : 2-*Di*-(2-*mercaptoethylthio*)*ethane.*—1 : 2-Di-(2-chloroethylthio)ethane (22 g.) and thiourea (16 g.) were refluxed in ethanol (80 c.c.) for 2 hours. The solvent was distilled off and the white residue dissolved in water (200 c.c.). A solution of sodium hydroxide (8 g.) in water (30 c.c.) was added and the mixture heated for 10 minutes at 100°. On cooling, the oily product solidified

and was filtered off and recrystallised from benzene–light petroleum. 1 : 2-*Di*-(2-*mercaptoethylthio*)*ethane* formed small white plates, m. p. 46° (Found : C, 33·2; H, 6·7. $C_6H_{14}S_4$ requires C, 33·6; H, 6·5%).

(*Diethyl Sulphone*)-2 : 2′-*bis-sulphonyl Chloride.*—(Diethyl sulphide)-2 : 2′-bisthiuronium chloride (4 g.) was dissolved in water (50 c.c.), and a slow stream of chlorine passed in at 0° until no more precipitate formed. The crude sulphonyl chloride was filtered off, washed with ice-water, and dried in a vacuum. As the material could not be recrystallised satisfactorily it was characterised by conversion into the dianilide by heating it at 100° with an excess of aniline, treating the mixture with dilute hydrochloric acid, and collecting the solid. Recrystallisation from 2-ethoxyethanol gave (*diethyl sulphone*)-2 : 2′-*disulphonanilide* as small plates, m. p. 220—221° (Found : N, 6·6. $C_{16}H_{20}O_4N_2S_3$ requires N, 6·7%). The diamide was also prepared by grinding the sulphonyl chloride with aqueous ammonia (*d* 0·88) and recrystallising the product from water, giving (diethyl sulphone)-2 : 2′-disulphonamide, m. p. 208—210°. The thiuronium salts from diethyl sulphoxide and diethyl sulphone were treated with chlorine as above, and in both cases mixed m. p.s showed that the same dianilide and diamide were obtained.

The greater part of this work was done in No. 2 Anti-Gas Laboratory R.E., during 1944, and it was completed at the Chemical Defence Experimental Establishment, Porton. The author is indebted to the Chief Scientist, Ministry of Supply, for permission to publish these results and to Mr. J. H. R. Slade for much experimental assistance and for the determination of molecular weights.

University Chemical Laboratory, Cambridge. [*Received, October 3rd,* 1951.]

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32. *Structure and Amœbicidal Activity. Part II.* Aliphatic Diamines.*

By D. Muriel Hall, Sardar Mahboob, and E. E. Turner.

7 : 13-Diamino-5 : 15-diethylnonadecane dihydrochloride has been synthesised and found to possess amœbicidal activity.

Previous work (*J.*, 1950, 1842) has been extended by the synthesis of 7 : 13-diamino-5 : 15-diethylnonadecane (dihydrochloride), by use of the Schmidt reaction on the corresponding 7 : 13-dicarboxy-compound, obtained by a malonic ester synthesis. The hydrochloride was active at a concentration of 1 in 500,000 against *Entamœba histolytica* (*in vitro* test by Dr. J. D. Fulton).

A second route to diamines was examined. By the action of cadmium alkyls on adipoyl chloride it is possible to prepare diketones of the type R·CO·$[CH_2]_4$·CO·R, whence diamines are obtainable by reducing the diketone dioximes. In this way 5 : 10-diaminotetradecane (dipicrate) was synthesised. When, however, an attempt was made to employ ββ-dialkylglutaric acids instead of adipic acid as starting material, the synthesis broke down, since we were unable to prepare 2 : 2-dimethylglutaroyl chloride; the acid was converted mainly into the anhydride by thionyl chloride.

A third route tried was from 2 : 2-dialkylpropane-1 : 3-diols, obtainable by reducing ethyl dialkylmalonates with ethereal lithium aluminium hydride. Conversion of 2 : 2-dimethylpropane-1 : 3-diol into the corresponding 1 : 3-dibromide, and condensation of the latter with ethyl *n*-amylmalonate, followed by a normal series of reactions, led to 6 : 10-diamino-8 : 8-dimethylpentadecane (dihydrochloride). This was active at 1 : 1000 against *E. histolytica*.

Experimental

Ethyl 5 : 15-*Diethylnonadecane*-7 : 7 : 13 : 13-*tetracarboxylate.*—Pentamethylene dibromide (76 g., 0·33 mole) was added to a solution of ethyl 2-ethylhexylmalonate (180 g., 0·7 mole) in absolute ethyl alcoholic sodium ethoxide (sodium, 14·95 g., 0·65 g.-atom). The mixture was

* Part I, *J.*, 1950, 1842.

stirred and boiled under reflux for 8 hours. The bulk of the alcohol was distilled off, water was added, and the cooled residue was extracted with ether. The ethereal solution was dried (Na_2SO_4) and the ether distilled off. The *ester* (yield 35%) boiled at 256—257°/4 mm. (Found: C, 69·6; H, 10·7. $C_{35}H_{64}O_8$ requires C, 68·6; H, 10·5%).

7 : 13-*Diamino*-5 : 15-*diethylnonadecane*.—Hydrolysis of the tetracarboxylic ester, followed by half-decarboxylation, gave a product which could not be crystallised. It was dissolved in 50 c.c. of benzene, and 25 c.c. of concentrated sulphuric acid were added. To the mixture sodium azide (2·5 g.) was added at 30—45°, followed by ice and then 30% sodium hydroxide solution. The mixture was extracted with benzene–ether, and the extracts were dried (Na_2SO_4). The solution obtained was used for making the *dihydrochloride* (Found: Cl, 16·4. $C_{23}H_{50}N_2,2HCl$ requires Cl, 16·5%) and the *dipicrate*, m. p. 142—144° (from aqueous alcohol) (Found: C, 50·3; H, 6·9; N, 13·8. $C_{35}H_{56}O_{14}N_8,H_2O$ requires C, 50·5; H, 7·0; N, 13·2%).

Tetradecane-5 : 10-*dione*.—This was prepared from di-*n*-butylcadmium and adipoyl chloride (cf. Cason and Prout, *J. Amer. Chem. Soc.*, 1944, **66**, 46; Burnett and Tarbell, *ibid.*, 1945, **67**, 1944; Cason, *Chem. Reviews*, 1947, **40**, 15). The crude diketone was converted into the *dioxime* which after being crystallised from benzene had m p. 117—119° (Found: C, 66·8; H, 11·0; N, 10·7. $C_{14}H_{28}O_2N_2$ requires C, 65·5; H, 11·0; N, 10·9%). Reduction of the dioxime with sodium and absolute alcohol, etc., led to 5 : 10-*diaminotetradecane dipicrate*, m. p. 205—207°, which was crystallised from alcohol (Found: C, 45·8; H, 5·3; N, 16·2. $C_{26}H_{38}O_{14}N_8$ requires C, 45·4; H, 5·5; N, 16·3%).

2 : 2-*Diethylpropane*-1 : 3-*diol*.—This diol was (95%) obtained by reduction of ethyl diethylmalonate with ethereal lithium aluminium hydride. It had b. p. 112°/5 mm., 118°/8 mm., and when crystallised from benzene had m. p. 65° (Found: C, 63·1; H, 12·0. Calc. for $C_7H_{16}O_2$: C, 63·5; H, 12·1%). It has previously been made by condensation of 2-ethylbutaldehyde and formaldehyde (Shortridge, Craig, Greenlee, Derfer, and Boord, *J. Amer. Chem. Soc.*, 1948, **70**, 946; McKusick, *ibid.*, p. 1982). The diol was treated with phosphorus tribromide but the resulting 1 : 3-dibromo-2 : 2-diethylpropane proved unsatisfactory for further synthetic work.

6 : 10-*Diamino*-8 : 8-*dimethylpentadecane Dihydrochloride*.—Condensation of 1 : 3-dibromo-2 : 2-dimethylpropane with an alcoholic solution of sodium ethoxide and ethyl *n*-amylmalonate gave a low yield of ethyl 8 : 8-dimethylpentadecane-6 : 6 : 10 : 10-tetracarboxylate. Hydrolysis, followed by half-decarboxylation and application, as above, of the Schmidt reaction, gave 6 : 10-*diamino*-8 : 8-*dimethylpentadecane dihydrochloride* as a deliquescent solid (Found: C, 60·1; H, 11·4; Cl, 19·7. $C_{17}H_{40}N_2Cl_2$ requires C, 59·4; H, 11·7; Cl, 20·6%).

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Bedford College, University of London. [*Received, October 9th*, 1951.]

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33. *Examples of the Direct Iodination of Aromatic Compounds.*

By I. R. L. Barker and William A. Waters.

Examples are given of the direct iodination of aromatic liquids by treatment with iodine in the presence of silver sulphate dissolved in concentrated sulphuric acid. For the iodination of benzene the conditions for attaining optimum yields are indicated. Iodination of naphthalene has been effected by using its solutions in *cyclo*hexane, but when high concentrations of sulphuric acid are used αα′-dinaphthyl is also formed.

Derbyshire and Waters (*J.*, 1950, 3694) have recently indicated the practicability of iodinating aromatic compounds by means of iodine and silver sulphate in the presence of sulphuric acid, and exemplified their procedure by describing the direct *m*-iodination of benzoic acid. We now report the successful iodination of several aromatic liquids by the same method. The concentration of sulphuric acid used is of prime importance in determining the percentage yield of the iodinated product. As the sulphuric acid is diluted the yield drops, probably on account of increasing hydrolysis of the iodine to a

mixture of iodide and iodate, though to some extent the diminished solubility of the aromatic compound in the aqueous phase may be a contributory factor.

Conversely, if the sulphuric acid concentration is too high, then the yield of iodo-compound may be impaired owing to concurrent sulphonation or other side reactions. These factors have been made evident by a study of the iodination of benzene and of toluene. Chlorobenzene and iodobenzene, which are somewhat less reactive, are both iodinated in high yield without difficulty.

As already indicated, the iodination process is satisfactory for some *meta*-substituting compounds. It is successful with benzaldehyde, nitrobenzene, and *p*-nitrotoluene, but it fails with *m*-dinitrobenzene and 2 : 4-dinitrotoluene, even at 100°, though both the latter compounds can be brominated successfully by the analogous procedure (Derbyshire and Waters, *J.*, 1950, 564). This result can be expected since the iodine cation should be a less potent electrophilic substituting agent than the bromine cation.

When attempts were made to extend the method to the direct iodinations of solid aromatic compounds insoluble in water, it was found that acetic acid, which had been used for the bromination of benzoic acid, was not always a practicable co-solvent, since on warming it reacted itself with the iodinating reagent. For the direct iodination of naphthalene a two-phase system comprising a solution of silver sulphate in sulphuric acid and a solution of naphthalene in *cyclo*hexane has been used with some success. With such mixtures, best yields of α-iodonaphthalene were obtained by use of 50—60% sulphuric acid; for when the concentration of sulphuric acid was raised to 75—85%, αα′-dinaphthyl formed the main product, and with over 85% acid sulphonation of the naphthalene occurred extensively.

This may be due to oxidation by the silver salt since αα′-dinaphthyl can be obtained in small yield by stirring a *cyclo*hexane solution of naphthalene with 85% sulphuric acid and silver sulphate, though the addition of α-iodonaphthalene considerably increases the yield. In the absence of silver sulphate naphthalene is removed from *cyclo*hexane solution by 85% sulphuric acid on account of sulphonation without any concurrent formation of dinaphthyl.

From the preparative standpoint, the utility of this method of iodination should be assessed on the assumption that complete recovery of the silver salt is achieved, and fortunately the quantitative collection of silver in the form of silver halides presents no difficulty. Our work indicates, however, that predictable factors, such as solubilities in sulphuric acid and liability to hydrolysis and/or sulphonation, severely limit the applicability of this method of iodination to further aromatic compounds and consequently it is of much less general value than the corresponding bromination method.

Experimental

Iodination of Benzene.—In a 1-l. 3-necked flask, fitted with a thermometer and a water-sealed stirrer, benzene (23 g., 0·3 mole) was rapidly stirred into an emulsion with a solution of silver sulphate (31 g., 0·1 mole) in a mixture of concentrated sulphuric acid (200 c.c.) and water (20 c.c.). Finely powdered iodine (56 g., 0·22 mole) was added in portions during 2 hours. After a further hour's stirring the mixture was diluted with water (600 c.c.) and decanted through a filter from the solid residue of silver iodide and free iodine. This residue was stirred for some time with an excess of sodium sulphite solution and again separated. The solid and the liquid portion of the reaction product were then extracted separately with ether, and after drying and removal of free iodine (Na_2SO_3) the organic product was fractionated. The yield of iodobenzene, b. p. 188°, which was collected under reduced pressure, was 32 g. (78% calc. on the silver sulphate, 98% of which was directly recovered as silver iodide and the small remainder precipitated as the chloride).

Yields obtained under other conditions were as follows :

H_2SO_4, concn. by vol., %	95	90	50	25	2N-HNO_3
C_6H_5I, calc. on Ag_2SO_4, %	75	80	40	0	0

Iodination of Toluene.—Toluene (14 g., 0·15 mole) was stirred with a solution of silver sulphate (15·6 g., 0·05 mole) in sulphuric acid (100 c.c.) and water (20 c.c.), and iodine (28 g.,

0·11 mole) was added during 1½ hours. On working up as described above, 12 g. (55%) of yellow oil, b. p. 205—215°, were obtained, consisting of mixed *o*- and *p*-iodotoluene. From this, by oxidation with aqueous potassium permanganate, there was obtained *p*-iodobenzoic acid, m. p. 266° (Whitmore and Woodward, *Org. Synth.*, 1927, **7**, 58, give 266—267°).

A similar yield of iodotoluenes was obtained by using 60% sulphuric acid by volume, but acids of over 80% concentration gave little product since sulphonation was too rapid.

Iodination of Chlorobenzene.—This was carried out similarly on the 0·05-mole scale by using 80% sulphuric acid by volume and gave 72% of crude iodination products from which was separated, by crystallisation from alcohol, 60% of *p*-chloroiodobenzene of m. p. 54—55° (the m. p. being unchanged after admixture with an authentic specimen prepared from *p*-chloroaniline), together with a little oil, b. p. 233°, which was probably the *o*-isomer.

Iodination of Iodobenzene.—Procedure identical with that used for chlorobenzene yielded 75% of crude iodination products which on crystallisation gave 60% of *p*-di-iodobenzene, m. p. 130—131° (Datta and Chatterjee, *J. Amer. Chem. Soc.*, 1919, **41**, 293, give 129·4°), and a residual oil, b. p. 280°, containing the *o*-isomer.

Iodination of Benzaldehyde.—Freshly distilled benzaldehyde (25 g., 0·25 mole) was stirred rapidly with a solution of silver sulphate (0·05 mole) dissolved in 80% sulphuric acid (100 c.c. of concentrated acid plus 20 c.c. of water) in a flask immersed in a water-bath kept at 65—70°. Iodine powder (27 g., 0·105 mole) was added during 1½ hours and thereafter the stirring was continued for a further 1½ hours. From time to time the reaction vessel was shaken vigorously to dislodge iodine which had sublimed above the liquid. On working up as usual, 10·4 g. (45%) of crude solid product was obtained which after repeated crystallisation gave 7 g. of *m*-iodobenzaldehyde of m. p. 58° (Patterson, *J.*, 1896, **89**, 1002, gives 57°), from which were prepared the semicarbazone, m. p. 226° (Willgerodt and Rieke, *Ber.*, 1905, **38**, 1479, give 225—226°), and the phenylhydrazone, m. p. 156° (Patterson, *loc. cit.*, gives 155°).

Iodination of Nitrobenzene.—Nitrobenzene (18·5 g., 0·15 mole) was stirred at 100° with a solution of silver sulphate (0·05 mole) in a mixture of concentrated sulphuric acid (110 c.c.) and water (15 c.c.), and iodine powder (0·105 mole) was added gradually during an hour. After a further hour's heating with stirring and occasional vigorous shaking (see above), the mixture was cooled and diluted with water. The organic layer was separated and, after removal of the excess of nitrobenzene by distillation under reduced pressure, yielded 15 g. (60%) of an oil which remained solid after freezing. After crystallisation from alcohol it had m. p. 34—39°; subsequent chromatographic separation on alumina, with benzene–light petroleum as the eluent, gave pure *m*-iodonitrobenzene (14 g., 55%), m. p. 38—39° unchanged by admixture with an authentic specimen prepared from *m*-nitroaniline.

The reaction occurred more slowly at 80°, but the yield (13·9 g.) was almost the same; at 60° very little iodination occurred.

Iodination of p-*Nitrotoluene.*—This was carried out at 80° on the 0·05-mole scale by the method used for nitrobenzene. The solid product was steam-distilled to remove unchanged *p*-nitrotoluene, and the residue (yield 60%), m. p. 45—54°, was repeatedly crystallised from methanol, whereupon pure 2-iodo-4-nitrotoluene, m. p. 53—54°, was obtained (Found: I, 48·1, 48·2. Calc. for $C_7H_6O_2NI$: I, 48·2%) (Willgerodt and Kok, *Ber.*, 1908, **41**, 2077, give m. p. 58°).

Similar iodinations were attempted on a boiling water-bath with both *m*-dinitrobenzene and 2 : 4-dinitrotoluene, but in neither case did any reaction occur.

Iodination of Naphthalene.—(a) *With 85% sulphuric acid.* A solution of naphthalene (12·8 g., 0·1 mole) in *cyclo*hexane (130 c.c.) was stirred at room temperature with a solution of silver sulphate (0·05 mole) in a mixture of concentrated sulphuric acid (130 c.c.) and water (25 c.c.). Iodine powder (0·105 mole) was added during an hour and stirring was continued for a further hour. The reaction mixture was worked up as usual, and the organic portion on fractionation under reduced pressure gave, besides unchanged naphthalene, 1·7 g. of an oil, b. p. *ca.* 300°/1 atm., and 4 g. of a solid residue which could be sublimed. 1·1 G. of this residue were dissolved in light petroleum and chromatographed on alumina with benzene–light petroleum mixtures. It yielded 1·1 g. of pure αα′-dinaphthyl, m. p. (and mixed m. p. with an authentic specimen) 157—158° (Willgerodt and Schlosser, *Ber.*, 1900, **33**, 698, give 158·5°). Similar material was obtained by sublimation. The oil when treated in alcohol with picric acid yielded the solid yellow picrate, m. p. 127·5°, of α-iodonaphthalene (Roux, *Bull. Soc. chim.*, 1886, **45**, 517, gives 127°).

(b) *With 50% sulphuric acid.* A reaction carried out on the above scale, but with 250 c.c. each of concentrated sulphuric acid and water, gave 8·6 g. (34%) of α-iodonaphthalene and

only 0·8 g. of tarry residue which after crystallisation from alcohol had m. p. 98—104° and contained iodine. Other mixtures gave the following yields :

H_2SO_4, % by vol.	85	75	60	50
α-Iodonaphthalene, %	6·7	3·1	32	34
αα′-Dinaphthyl, %	34·5	33·5	0	0

Reaction between Naphthalene and α-Iodonaphthalene.—Silver sulphate (4·5 g.) dissolved in a mixture of sulphuric acid (85 c.c.) and water (15 c.c.) was stirred for 1½ hours with a solution of naphthalene (10 g.) and α-iodonaphthalene (6·4 g.) in *cyclo*hexane (80 c.c.), during which time it gradually became black. The organic material was then separated and steam-distilled to remove the excess of naphthalene, and the residue was fractionated under reduced pressure. There were obtained 1·4 g. of a dark solid which on chromatographic purification yielded 1·09 g. of αα′-dinaphthyl, m. p. and mixed m. p. 157—158°.

In a similar experiment in which a solution of 10 g. of naphthalene in 100 c.c. of *cyclo*hexane was stirred with a solution of 8 g. of silver sulphate in 85 c.c. of sulphuric acid and 15 c.c. of water there was obtained 0·37 g. of αα′-dinaphthyl. When no silver sulphate was present an identical reaction mixture, after 1½ hours' stirring, contained only 5·5 g. of naphthalene and left no residue after steam-distillation. When a mixture of 90 c.c. of sulphuric acid and 10 c.c. of water was used as the solvent, the whole of the naphthalene originally present in a similar *cyclo*hexane solution was removed during 2 hours' stirring at room temperature, forming sulphonic acids which dissolved in the aqueous layer.

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34. *Researches in the Menthone Series. Part XVII.* Configurations of Menthols and Menthylamines.*

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Each of the four stereoisomeric menthyltrimethylammonium hydroxides on thermal decomposition affords one of three *p*-menthenes. Menthyl- and *iso*menthyltrimethylammonium hydroxides give isomeric *p*-menth-2-enes which on hydrogenation yield isomeric *p*-menthanes. Application of von Auwers's rule to the physical properties of these *p*-menthanes indicates that menthone and its derivatives have the *trans*-configuration on carbon atoms 1 and 4 while *iso*menthone and its derivatives have the *cis*-configuration. Since quaternary ammonium hydroxides have been shown to decompose by a bimolecular mechanism dependent wholly on *trans*-elimination, additional evidence for the accepted configurations on carbon atoms 3 and 4 of the four menthylamines, and hence also of the four menthols, is now presented (cf. Read and Grubb, *J.*, 1934, 1779). *neoiso*Menthylamine has been prepared by a new method. New optical constants have been calculated for (+)-*trans*- and (+)-*cis*-*p*-menth-2-ene.

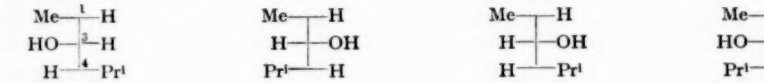
THE methyl and *iso*propyl groups on carbon atoms 1 and 4 respectively of menthone and *iso*menthone have been assigned the *trans*- and the *cis*-configuration respectively by applying the rule of von Auwers (*Annalen*, 1920, **420**, 92) to their physical properties and to those of their derivatives. Zeitschel and Schmidt (*Ber.*, 1926, **59**, 2298), using optically pure materials, found that (+)-*iso*menthone had a higher density and refractive index than (−)-menthone. This was confirmed by Hiraidzumi (Chikashige Anniv. Vol. Kyoto, 1930, 87). If von Auwers's rule is assumed to hold for cyclic compounds, *iso*menthone should be assigned the *cis*- and menthone the *trans*-configuration. Additional evidence for these configurations was presented by Keats (*J.*, 1937, 2003) who converted (−)-menthone and (±)-*iso*menthone into the corresponding menthanes by electrolytic reduction using Scholl and Kirst's procedure (*Z. Electrochem.*, 1923, **29**, 537). (±)-*iso*Menthone gave the menthane having the higher density and refractive index : as before, this indicates a

* Part XVI, *J.*, 1939, 1306.

cis-configuration. Hückel and his co-workers (*Annalen*, 1939, **537**, 126; *Ber.*, 1941, **74**, 657) prepared *p*-menth-2-enes by treating (−)-menthyl and (+)-*iso*menthyl toluene-*p*-sulphonate with alcoholic sodium ethoxide. In this case also the menthene derived from the *iso*menthyl compound had the higher density and refractive index, indicating that it and the related *iso*menthone should be assigned the *cis*-configuration.

Read and Grubb (*loc. cit.*) used the comparative rates of esterification of the menthols with *p*-nitrobenzoyl chloride to determine the configurations of the groups on carbon atom 3. They found that the two *neo*menthols esterified much more slowly than either menthol or *iso*menthol. On the assumption that steric hindrance by the *iso*propyl group is the main factor adverse to esterification, the hydroxyl group on carbon atom 3 is assigned the *cis*-position relative to the *iso*propyl group in the two *neo*menthols, and the *trans*-position in menthol and *iso*menthol.

Since (−)-menthol and (+)-*neo*menthol have been related configurationally to (−)-menthone (Zeitschel and Schmidt, *Ber.*, 1926, **59**, 2303), and since (+)-*iso*menthol and (+)-*neoiso*menthol have been related to (+)-*iso*menthone (Read, Robertson and Cook, *J.*, 1927, 1276; Read and Grubb, *J.*, 1934, 313), the relative configurations of the menthols can be written as shown :



(−)-Menthol (+)-*iso*- (+)-*neo*- (+)-*neoiso*-

The four menthylamines have been assigned corresponding configurations partly on the basis of similarities of values for optical rotation between the menthylamine and the menthols (Read and Grubb, *J.*, 1934, 314). Attempts to use the rate of formation of acyl derivatives of the amines as a criterion for the relative configurations on carbon atoms 3 and 4 led to anomalous results (Read and Grubb, *J.*, 1934, 1779).

We now present additional evidence in favour of the above configurations for the menthols and the menthylamines. Optically active menthenes were prepared from each of the four menthylamines by way of the quaternary ammonium hydroxides, Read and Hendry's method (*Ber.*, 1938, **71**, 2544) being used with some modifications. The menth-2-ene derived in this way from (+)-*iso*menthylamine had a higher density and a higher refractive index than that derived from (−)-menthylamine. By von Auwers's rule, the *cis*-configuration of carbon atoms 1 and 4 is accordingly assigned to (+)-*iso*menthylamine, as also to the related (+)-*iso*menthone, and the *trans*-configuration to (−)-menthylamine and (−)-menthone. This result confirms the conclusion drawn from the work of Hückel and his co-workers (*loc. cit.*), who prepared the isomeric menth-2-ene by a different method.

Since von Auwers's rule is applied with greater certainty when the disubstituted ring is saturated, the menth-2-enes prepared as above were hydrogenated to the corresponding *p*-menthanes. Again, the menthane derived from (+)-*iso*menthone had a higher density and a higher refractive index than that from (−)-menthone. This work, in which optically pure starting materials and purely chemical reactions were used throughout, confirms the results of Keats (*loc. cit.*), who used electrolytic reduction.

Recently it has been shown by Rossini and Pitzer (*Science*, 1947, 647) that the general application of von Auwers's rule to cyclic compounds is susceptible to error. In a study of the thermodynamic properties of isomeric 1 : 3-dimethyl*cyclo*hexanes they found that the *cis*-configuration is less strained and has a lower energy content than the *trans*-configuration. Accordingly they assigned the *cis*-configuration to the lower-boiling isomer, which also had the lower density and refractive index. This is contrary to von Auwers's rule. Mousseron and Granger (*Bull. Soc. chim.*, 1938, **5**, 1618) arrived at the same conclusion from a study of the optically active forms of 1 : 3-dimethyl*cyclo*hexane.

However, Rossini and Pitzer concluded that the *trans*-1 : 2- and *trans*-1 : 4-dimethyl-*cyclo*hexanes should have the lower energy content and hence should follow von Auwers's rule. Since the menthanes are 1 : 4-disubstituted *cyclo*hexanes it is very probable that they also follow von Auwers's rule and that the configurations on carbon atoms 1 and 4 deduced for the menthones, menthylamines, and menthols are correct.

The identification of the menthenes prepared by pyrolysis of the four optically active quaternary ammonium hydroxides of the menthylamines gives additional evidence for the configuration of the groups on carbon atom 3. It has been established that quaternary ammonium hydroxides on thermal decomposition form olefins by a bimolecular ionic mechanism associated with *trans*-elimination (Dhar, Hughes, Ingold, Mandour, Maw, and Woolf, *J.*, 1948, 2117).

The appended table shows the composition, determined by Hückel's method (*Annalen*, **1940, 543, 191**), of the olefins formed by pyrolysis of each of the four quaternary ammonium hydroxides from the menthylamines :

Quaternary ammonium compound	(+)-*trans*-*p*-Menth-2-ene	(+)-*cis*-*p*-Menth-2-ene	(+)-*p*-Menth-3-ene
Menthyl	96	—	4
*iso*Menthyl	—	99	1
*neo*Menthyl	10·5	—	89·5
*neoiso*Menthyl	—	present	mainly

The accepted relative configurations for the four quaternary ammonium ions are :

(−)-Menthyl- (+)-*iso*- (+)-*neo*- (+)-*neoiso*-

Examination of these formulæ shows that with the menthyl and *iso*menthyl compounds *trans*-elimination can occur only between carbon atoms 2 and 3. This has been found to be substantially the case, since very little *p*-menth-3-ene is formed. With the *neo*menthyl and the *neoiso*menthyl compounds *trans*-elimination can occur either between carbon atoms 3 and 4 or between atoms 2 and 3. Elimination occurs by both routes, but in each case elimination predominates between carbon atoms 3 and 4, towards the tertiary carbon.

Since these results fit in well with the accepted configuration for carbon atom 3 it can be concluded that these configurations of the quaternary ammonium compounds, the menthylamines, and the menthols are probably correct.

Experimental

(M. p.s are uncorrected. Analyses are by Drs. Weiler and Strauss, Oxford.)

Menthyltrimethylammonium Hydroxide.—(−)-Menthol (45 g.), $[\alpha]_D^{16}$ −48·9° (*c*, 3·83 in ethanol), was converted by Beckmann's method (*Annalen*, 1889, **250**, 325) into (−)-menthone (42 g., 95%), α_D^{15} −25·62° (*l* = 1 dm.; homogeneous). (−)-Menthone (332 g.), α_D^{16} −23·9° (*l* = 1 dm.; homogeneous), from several similar preparations, with hydroxylamine acetate in ethyl alcohol at room temperature gave a crystalline oxime (267 g., 73%), m. p. 49—50°, $[\alpha]_D^{15}$ −42·0° (*c*, 2·47 in ethanol), and an oily oxime (79 g., 22%), $[\alpha]_D^{18}$ −36·6° (*c*, 2·48 in ethanol). The crystalline oxime (112 g.) was reduced by sodium and ethanol (Wallach, *Annalen*, 1893, **276**, 301) to (−)-menthylamine (58 g., 57%), α_D^{17} −31·51° (*l* = 1 dm.; homogeneous). The hydrochloride, after recrystallising once from water, had $[\alpha]_D^{11}$ −32·4° (*c*, 1·88 in water), and (−)-*menthylamine hydrobromide* had $[\alpha]_D^{11}$ −26·9° (*c*, 2·74 in water) (Found : Br, 33·8. $C_{10}H_{22}NBr$ requires Br, 33·9%). The salicylidene derivative was phototropic and had m. p. 57—58°, $[\alpha]_D^{13}$ −118·2° (*c*, 2·17 in chloroform).

The oily oxime (79 g.) was reduced by the same procedure to (−)-menthylamine (48 g., 66%), α_D^{17} −29·67° (*l* = 1 dm.; homogeneous). Its salicylidene derivative was phototropic and had m. p. 56—57°, $[\alpha]_D^{16}$ −119·9° (*c*, 1·99 in chloroform). No depression in m. p. was obtained when this derivative was mixed with the salicylidene derivative of authentic (−)-menthylamine.

Since both oximes give (−)-menthylamine on reduction they are probably *syn*- and *anti*-isomers. This type of isomerism has already been reported by Hückel and Doll (*Annalen*, 1936, **526**, 103) who isolated *syn*- and *anti*-forms of carvomenthone oxime and *iso*carvomenthone oxime.

(−)-Menthylamine (67 g.) was converted into (−)-menthyltrimethylammonium iodide (129 g., 93%) by the method of Read and Hendry (*Ber.*, 1938, **71**, 2544) except that a 50%

excess of methyl iodide was used to ensure higher yields. After recrystallising from acetone the methiodide had m. p. 193—194°, $[\alpha]_D^{17}$ −37·6° (*c*, 2·59 in water). Read and Hendry (*loc. cit.*) give m. p. 190° (decomp.), $[\alpha]_D^{17}$ −39·3° (*c*, 2 in water).

The methiodide (56 g.) in aqueous solution was converted into the quaternary ammonium hydroxide by treating it with excess of freshly prepared moist silver oxide. The precipitated silver iodide and excess of silver oxide were filtered off, leaving the menthyltrimethylammonium hydroxide in solution.

Pyrolysis of Menthyltrimethylammonium Hydroxide.—The above menthyltrimethylammonium hydroxide solution was concentrated on a steam-bath and then distilled under atmospheric pressure at a bath-temperature of 130—140°. Two fractions were recovered which on being washed with dilute hydrochloric acid, aqueous sodium carbonate, and water and dried (Na_2SO_4) gave colourless liquids: 2·3 g., α_D^{14} +107·87° (*l* = 1 dm.; homogeneous); and 1·8 g., α_D^{17} +99·34° (*l* = 1 dm.; homogeneous). A third fraction (3·0 g.), α_D^{17} +104·30° (*l* = 1 dm.; homogeneous), was obtained on continuing the distillation at 200° (bath-temp.)/20 mm. The overall yield of menthene was 7·1 g. (30%). After distillation of the combined fractions three times from sodium under a vacuum, (+)-*trans*-*p*-menth-2-ene having the following properties was obtained: α_D^{16} +107·18° (*l* = 1 dm.; homogeneous), $[\alpha]_D^{16}$ +132·5° (homogeneous), d_4^{20} 0·8096, n_D^{20} 1·4492, $[R_L]_D$ 45·78 (Calc., 45·71). As found by Read and Hendry (*loc. cit.*), the distillate also contained (−)-menthyldimethylamine which was removed in the acid wash.

Racemisation of (+)-trans-p-*Menth*-2-*ene*.—Hückel (*Annalen*, 1940, **543**, 191) has shown that on being refluxed with alcoholic toluene-*p*-sulphonic acid (+)-*p*-menth-3-ene and (+)-*p*-menth-1-ene are completely racemised, whereas (+)-*trans*- and (+)-*cis*-*p*-menth-2-ene are not affected. The above (+)-*trans*-*p*-menth-2-ene (0·6 ml.) was refluxed with toluene-*p*-sulphonic acid solution (0·8 ml.) (29·4 g. of p-$C_6H_4Me{\cdot}SO_3H,4H_2O$ in 100 ml. of ethanol solution) for 4·5 hours. After recovery by addition of water, washing with aqueous sodium carbonate, and drying (Na_2SO_4), the menthene had α_D^{18} +103·6° (*l* = 1 dm.; homogeneous). A series of racemisation tests on another sample of (+)-*trans*-*p*-menth-2-ene showed that little further drop in rotation occurred after 4 hours' refluxing. If the value α_D +88·0° is assumed for optically pure (+)-*p*-menth-3-ene, as calculated by Hückel (*loc. cit.*), the composition of the *p*-menthene having α_D^{18} +107·18° (*l* = 1 dm.; homogeneous) can be estimated by using the relation given by Landolt ("Optical Rotation of Organic Substances," 2nd. edn., 1902, p. 240). This was found to be (+)-*trans*-*p*-menth-2-ene 95·9 and (+)-*p*-menthene-3-ene 4·1%. By the same relation it can be estimated that optically pure (+)-*trans*-*p*-menth-2-ene should have α_D +108·0° (*l* = 1 dm.; homogeneous).

trans-p-*Menthane*.—(+)-*trans*-*p*-Menth-2-ene (2·7 g.), α_D^{18} +107·18° (*l* = 1 dm.; homogeneous), in glacial acetic acid (10 ml.), was hydrogenated at 4 atm. at room temperature over Adams's platinum oxide (16 mg.). The absorption stopped when one molecular equivalent of hydrogen had been taken up in 12 minutes. After the catalyst had been removed by filtration the *trans*-*p*-menthane was recovered by adding water, washing with aqueous sodium carbonate and with aqueous potassium permanganate, and drying (Na_2SO_4). It had α_D^{18} −0·38° (*l* = 1 dm.; homogeneous), d_4^{20} 0·7936, n_D^{20} 1·4366, $[R_L]_D$ 46·24 (calc., 46·18).

Dibromide of (+)-trans-p-*Menth*-2-*ene*.—(+)-*trans*-*p*-Menth-2-ene (1·57 g.), α_D^{18} +104·10° (*l* = 1 dm.; homogeneous), in glacial acetic acid (5 ml.), was titrated at room temperature with bromine in glacial acetic acid (3·27 g.-atoms per l.); 2·00 atomic equivalents of bromine were absorbed with liberation of heat. The dibromide was recovered by addition of water, washing with aqueous sodium carbonate, and drying (Na_2SO_4), to give (+)-2 : 3-dibromo-*trans*-*p*-menthene (2·74 g., 81%), α_D^{17} +60·70° (*l* = 1 dm.; homogeneous), $[\alpha]_D^{17}$ +41·4° (homogeneous), d_4^{20} 1·4686, n_D^{20} 1·5239, $[R_L]_D$ 62·07 (calc., 61·71). This dibromide was probably a mixture of stereoisomers.

Debromination of (+)-2 : 3-*Dibromo*-trans-p-*menthene*.—Debromination was carried out by Linnemann's procedure (*Ber.*, 1877, **10**, 1113). (+)-2 : 3-Dibromo-*trans*-*p*-menthane (2·74 g.), α_D^{17} +60·70° (*l* = 1 dm.; homogeneous), was refluxed with water (1 ml.), ethanol (7 ml.), and zinc dust (1·0 g.) for 3 hours. After filtration, washing with water, and drying (Na_2SO_4), the resulting (+)-*trans*-*p*-menth-2-ene had α_D^{18} +102·20° (*l* = 1 dm.; homogeneous).

neo*Menthyltrimethylammonium Hydroxide*.—(+)-Formyl*neo*menthylamine (180 g.), m. p. 116—118°, was converted by the method of Read and Robertson (*J.*, 1926, 2217) into *neo*-menthylamine (158 g., 100%). The hydrochloride, recrystallised twice from water, had $[\alpha]_D^{15}$ +18·9° (*c*, 1·78 in water). The free base, α_D^{15} +11·38° (l = 1 dm.; homogeneous), from this salt (100 g.) was converted into (−)-*neo*menthyltrimethylammonium iodide (160 g., 76%)

by the method used for (−)-menthylamine. After recrystallising from acetone it had m. p. 161—162°, $[\alpha]_D^{18}$ −20·6° (*c*, 3·35 in water). Read and Hendry (*loc. cit.*) reported m. p. 160·5° (decomp.), $[\alpha]_D^{17}$ −19·5° (*c*, 2 in water). The quaternary ammonium hydroxide was prepared by the method used for the menthyl compound.

Pyrolysis of neo*Menthyltrimethylammonium Hydroxide.*—The quaternary ammonium hydroxide from 40 g. of the above iodide was distilled initially at atmospheric pressure and 120° (bath-temp.) and finally at 180°/20 mm. The greater part of the colourless oily distillate came over at the lower temperature. On working up of the distillate and distillation from sodium, (+)-*p*-menth-3-ene (86%) was obtained having α_D^{16} +90·09° (*l* = 1 dm.; homogeneous), $[\alpha]_D^{16}$ +110·8° (homogeneous), d_4^{20} 0·8138, n_D^{20} 1·4519, $[R_L]_D$ 45·79 (calc., 45·71). No tertiary amine was found in the distillate from this pyrolysis. The nitrosochloride, prepared by the method of Richtman and Kremers (*Amer. Chem. J.*, 1896, **18**, 762), was obtained in 41% yield as a colourless crystalline solid. After three recrystallisations from ethanol–chloroform it had m. p. 124—126°, $[\alpha]_D^{19}$ +226° (*c*, 1·8 in benzene) [Found: *M* (cryoscopic in benzene), 415. Calc. for $(C_{10}H_{18}ONCl)_2$: *M*, 407].

Racemisation of (+)-p-*Menth*-3-*ene.*—The above (+)-*p*-menth-3-ene was subjected to a series of racemisation tests with alcoholic toluene-*p*-sulphonic acid by Hückel's method (*loc. cit.*). The rotation dropped in 5·75 hours to a constant value of α_D^{17} +11·26° (*l* = 1 dm.; homogeneous). The composition of this menthene is estimated by the previously described method to be (+)-*p*-menth-3-ene 89·5 and (+)-*trans*-*p*-menth-2-ene 10·5%.

Hydrogenation of (+)-p-*Menth*-3-*ene.*—(+)-*p*-Menth-3-ene (4·33 g.), α_D^{16} +90·08° (*l* = 1 dm.; homogeneous), was hydrogenated by the method used for (+)-*trans*-*p*-menth-2-ene. Absorption of hydrogen stopped after one molecular equivalent had been added. The resulting *p*-menthane (4·3 g., 99%), after distillation over sodium at 15 mm., had α_D^{17} −0·06° (*l* = 1 dm.; homogeneous), d_4^{20} 0·7996, n_D^{20} 1·4407, $[R_L]_D$ 46·26 (calc., 46·18). This *p*-menthane is estimated to contain *cis*-*p*-menthane 55 and *trans*-*p*-menthane 45%, on the assumption of a linear variation of density and refractive index with concentration.

Dibromide of (+)-p-*Menth*-3-*ene.*—(+)-*p*-Menth-3-ene (1·0 g.), α_D^{16} +90·08° (*l* = 1 dm.; homogeneous), was brominated by the procedure used for (+)-*trans*-*p*-menth-2-ene: 1·94 atomic equivalents were absorbed with evolution of heat. The dibromide (1·95 g., 90%) had α_D^{18} +35·87° (*l* = 1 dm.; homogeneous), $[\alpha]_D^{18}$ +24·8° (homogeneous), d_4^{20} 1·4443, n_D^{20} 1·5260, $[R_L]_D$ 63·32 (calc., 61·71). This dibromide was probably a mixture of stereoisomers.

Debromination of (+)-p-*Menth*-3-*ene Dibromide.*—The above dibromide was debrominated by Linnemann's method (*loc. cit.*). The resulting *p*-menth-3-ene had $[\alpha]_D^{18}$ +28·1° (*c*, 2·99 in ethanol).

iso*Menthyltrimethylammonium Hydroxide.*—(−)-Piperitone (500 ml.), α_D^{16} −41·22° (*l* = 1 dm.; homogeneous), was hydrogenated by the method of Hughesdon, Smith, and Read (*J.*, 1923, **123**, 2921), using colloidal palladium as catalyst. Absorption was complete after 18 hours when one molecular equivalent of hydrogen had been added. After steam-distillation and drying (Na_2SO_4), (+)-*iso*menthone (467 g., 99%), α_D^{17} +49·0° (*l* = 1 dm.; homogeneous) was recovered. This was converted into the oxime by the method used for (−)-menthone. (+)-*iso*Menthone oxime (525 g., 100%), $[\alpha]_D^{18}$ +25·0° (*c*, 5·76 in ethanol), was obtained as an oil. The oxime (121 g.) was reduced with sodium and ethyl alcohol, by the method used for (−)-menthone oxime, to (+)-*iso*menthylamine (89 g., 80%), α_D^{15} +12·10° (*l* = 1 dm.; homogeneous). This was converted into the salicylidene derivative (Read and Robertson, *J.*, 1926, 2221), which after recrystallising three times from ethyl alcohol attained the constant rotation $[\alpha]_D^{15}$ +78·6° (*c*, 2·16 in chloroform). Hydrolysis of this derivative (118 g.) by refluxing it with aqueous sulphuric acid using the procedure of Read and Robertson (*J.*, 1927, 2171) afforded (+)-*iso*menthylamine (64 g., 100%). This was converted by the method used for (−)-menthylamine into (+)-*iso*menthyltrimethylammonium iodide (139 g., 100%). After two recrystallisations from acetone it formed long needles with the constant properties: m. p. 185°, $[\alpha]_D^{16}$ +0·6° (*c*, 3·46 in water). Read and Cuthbertson (*Rec. Trav. chim.*, 1950, **69**, 539) reported m. p. 184°, $[\alpha]_D^{18}$ +2·0° (*c*, 1·5 in water). The methiodide was converted into *iso*menthyltrimethylammonium hydroxide by moist silver oxide as described previously.

Pyrolysis of iso*Menthyltrimethylammonium Hydroxide.*—The quaternary ammonium hydroxide prepared from the iodide (40 g.) was distilled at 20 mm. and a maximum bath-temperature of 200°. An oil started to distil at a bath-temperature of 145°. After being washed with dilute hydrochloric acid and then aqueous sodium carbonate and dried (Na_2SO_4), (+)-*cis*-*p*-menth-2-ene (9·9 g., 58%) was obtained. After distilling from sodium at 15 mm. it had α_D^{16} +37·98° (*l* = 1 dm.; homogeneous), $[\alpha]_D^{16}$ +46·5° (homogeneous), d_4^{20} 0·8187, n_D^{20} 1·4530,

$[R_L]_D$ 45·61 (calc., 45·71). Besides the menthene there was obtained an oily base (4·6 g., 20%), α_D^{18} +38·64° (*l* = 1 dm.; homogeneous), probably *iso*menthyldimethylamine.

Racemisation of (+)-cis-p-*Menth*-2-ene.—A series of racemisation tests on the above menthene using Hückel's method (*loc. cit.*) showed that a constant value of α_D^{17} +37·20° (*l* = 1 dm.; homogeneous) was reached in 4 hours. This indicates that the menthene contains (+)-*cis*-*p*-menth-2-ene 99 and (+)-*p*-menth-3-ene 1%, and that optically pure (+)-*cis*-*p*-menth-2-ene has α_D^{17} +37·5° (*l* = 1 dm.; homogeneous).

Hydrogenation of (+)-cis-p-*Menth*-2-*ene*.—(+)-*cis*-*p*-Menth-2-ene (2·91 g.), α_D^{16} +37·98° (*l* = 1 dm.; homogeneous), was hydrogenated by the method used for (+)-*trans*-*p*-menth-2-ene. Absorption of hydrogen stopped after one molecular equivalent had been taken up. *cis*-*p*-Menthane (2·6 g., 88%) was obtained, which on distillation over sodium had α_D^{18} ±0·00° (*l* = 1 dm.; homogeneous), d_4^{20} 0·8047, n_D^{20} 1·4421, $[R_L]_D$ 46·10 (calc. 46·18).

Dibromide of (+)-cis-p-*Menth*-2-*ene*.—(+)-*cis*-*p*-Menth-2-ene (1·9 g.), α_D^{16} +37·98° (*l* = 1 dm.; homogeneous), was brominated by the procedure used for (+)-*trans*-*p*-menth-2-ene: 2·20 atomic equivalents of bromine were taken up with liberation of heat. The dibromide (4·08 g., 99%) had α_D^{18} −53·99° (*l* = 1 dm.; homogeneous), $[\alpha]_D^{18}$ −36·1° (homogeneous), d_4^{20} 1·4948, n_D^{20} 1·5299, $[R_L]_D$ 61·56 (calc., 61·71), b. p. 194°(decomp.)/760 mm. On debromination by the method used for (+)-2 : 3-dibromo-*trans*-*p*-menthane the resulting (+)-*cis*-*p*-menth-2-ene had α_D^{18} +31·85° (*l* = 1 dm.; homogeneous).

neoiso*Menthyltrimethylammonium Hydroxide*.—(+)-*iso*Menthone oxime (239 g.), α_D^{18} +25·7° (*c*, 5·47 in ethanol), in methyl alcohol (500 ml.) was hydrogenated at 106°/100 atm., 2 teaspoonsfuls of Raney nickel catalyst being used. The absorption of hydrogen stopped after 15 hours, when two molecular equivalents had been taken up. After steam-distillation an oily amine (58 g., 26%), α_D^{20} +2·01° (*l* = 1 dm.; homogeneous), was recovered from the distillate. An acid-insoluble oily ketone, α_D^{18} +3·82° (*l* = 1 dm.; homogeneous), made up the remainder of the distillate. The amine was converted into the salicylidene derivative, which after recrystallising seven times from light petroleum (b. p. 40—60°) to constant m. p. and rotation had m. p. 98—99°, $[\alpha]_D^{19}$ −19·5° (*c*, 2·43 in chloroform). No depression in m. p. was obtained when this derivative was mixed with authentic salicylidene*neoiso*menthylamine, prepared by the original method of Read and Robertson (*J.*, 1927, 2168). The salicylidene derivative (5·2 g.) was hydrolysed, giving *neoiso*menthylamine (2·3 g., 74%). This was converted by the method used for (−)-menthylamine into the methiodide, which after recrystallising once from acetone gave (+)-*neoiso*menthyltrimethylammonium iodide (2·9 g., 60%) having the constant properties: m. p. 195° (decomp.), $[\alpha]_D^{18}$ +16·5° (*c*, 1·88 in water) (Found: I, 39·4. Calc. for $C_{13}H_{28}NI$: I, 39·1%). Read and Cuthbertson (*loc. cit.*) reported m. p. 195°, $[\alpha]_D^{25}$ +16·8° (c, 1·5 in water). The quaternary ammonium hydroxide was prepared by the method used for the (−)-menthyl compound.

Pyrolysis of neoiso*Menthyltrimethylammonium Hydroxide*.—The quaternary ammonium hydroxide from 2·9 g. of iodide was distilled at 20 mm. and 195° (maximum bath-temp.). (+)-*p*-Menth-3-ene (0·16 g., 30%), $[\alpha]_D^{16}$ +94·1° (*c*, 1·39 in ethanol), was obtained along with an oily amine (0·30 g., 42%), α_D^{17} −13·34° (*l* = 1 dm.; homogeneous), probably *neoiso*menthyldimethylamine.

Racemisation of (+)-p-*Menth*-3-*ene*.—When the above menthene was refluxed for 4 hours with alcoholic toluene-*p*-sulphonic acid according to Hückel's procedure (*loc. cit.*), the rotation dropped to $[\alpha]_D^{16}$ +9·6° (*c*, 1·14 in ethanol). This low rotation, especially in view of the enhanced rotatory powers of such menthenes in ethanol (cf. Read and Robertson, *J.*, 1926, 2217), indicates that the menthene consisted essentially of (+)-*cis*-*p*-menth-2-ene with a little (+)-*cis*-*p*-menth-2-ene.

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United College of St. Salvator and St. Leonard,
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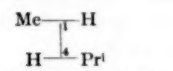
35. *Researches in the Carvone Series. Part V.* Configurations of Carvomenthones and Carvomenthylamines and their Steric Relation to Menthones.*

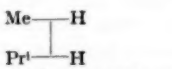
By Neal L. McNiven and John Read.

Carvomenthyltrimethylammonium hydroxide prepared from (−)-carvomenthone gives on pyrolysis (−)-*trans*-*p*-menth-2-ene of high optical purity. This result is used to supply further evidence for the accepted configurations of the carvomenthones, carvomenthylamine, and *neo*carvomenthylamine, and to establish the stereochemical relationship between the menthone and carvomenthone series. By relating (−)-menthone and (−)-carvomenthone to the same optically active *trans*-*p*-menth-2-ene it has been established that (−)-menthone is related configurationally to (+)-carvomenthone.

Johnston and Read (*J.*, 1935, 1138) prepared optically pure carvomenthone and *iso*carvomenthone and determined some of their physical properties. Application of von Auwers's rule (*Annalen*, 1920, **420**, 92) led to the conclusion that (−)-carvomenthone had the *trans*-configuration (I), and the configurationally related (−)-*iso*carvomethone the *cis*-configuration (II) :



(I) (−)-Carvomenthone



(II) (−)-*iso*Carvomenthone

No further evidence has hitherto been forthcoming for the configurations of the carvomenthones; in this respect the carvomenthones have not been so fully investigated as the menthones.

Four optically active carvomenthols have been prepared (Johnston and Read, *loc. cit.*). (+)-Carvomenthol and (−)-*neo*carvomenthol have been well characterised and have been shown to be related to (−)-carvomenthone. The remaining pair, (−)-*iso*carvomenthol and (−)-*neoiso*carvomenthol, have not been characterised with as great certainty, but (−)-*iso*carvomenthol has been shown to be related to (−)-*iso*carvomenthone. Preliminary investigations by Johnston and Read showed that (+)-carvomenthol reacted more rapidly than (−)-*neo*carvomenthol with acid chlorides. If steric hindrance by the methyl group is the main factor deterring esterification, the hydroxyl group on carbon atom 2 must be assigned the *cis*-position to the methyl group in (−)-*neo*carvomenthol and the *trans*-position in (+)-carvomenthol.

Three of the corresponding carvomenthylamines have been prepared. The fourth, *neoiso*carvomenthylamine, has not yet been isolated. Hückel and Wilip (*J. pr. Chem.*, 1941, **158**, 21) showed that (−)-*neo*carvomenthylamine, prepared by catalytic hydrogenation of the oxime of (−)-carvomenthone, on treatment with nitrous acid gave a mixture of carvomenthols and a menthene. This menthene was found to contain (−)-*p*-menth-1-ene, racemised *p*-menthene, and 18% of a lævorotatory menthene resistant to racemisation by toluene-*p*-sulphonic acid. As Hückel and Tappe (*Annalen*, 1939, **537**, 126) have shown, the last menthene must be either *trans*- or *cis*-*p*-menth-2-ene. If, on the basis of von Auwers's rule, carvomenthone is considered to have the *trans*-configuration, the menthene in question must be (−)-*trans*-*p*-menth-2-ene. Since (−)-menthone leads to the formation of (+)-*trans*-*p*-menth-2-ene (McNiven and Read, foregoing paper), it follows that (−)-menthone must be related configurationally to (+)-carvomenthone.

The present investigation shows the stereochemical relations between menthone and carvomenthone without requiring the assumption of a *trans*-configuration for carvomenthone. In fact, it supplies additional evidence for the configuration of carvomenthone.

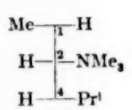
* Part IV, *J.*, 1937, 239.

In addition, a proof for the configuration of the groups on carbon atom 2 of carvomenthylamine and *neo*carvomenthylamine is provided.

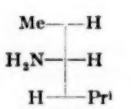
A menthene of high optical purity was prepared from (−)-carvomenthone *via* the quaternary ammonium hydroxide by using the method of Read and Hendry (*Ber.*, 1938, **71**, 2544) with some modifications. The *p*-menthene had $[\alpha]_D^{15}$ −139·5° (*c*, 1·9 in ethanol), which was unaffected by refluxing the hydrocarbon with toluene-*p*-sulphonic acid (showing the absence of active *p*-menth-1-ene). Read and Hendry (*loc. cit.*) reported the value $[\alpha]_D^{17}$ +149·7° (*c*, 1·6 in alcohol) for (+)-*trans*-*p*-menth-2-ene. Since no decrease in rotation occurred on treatment with toluene-*p*-sulphonic acid, the terpene concerned must be a *p*-menth-2-ene; moreover, since *cis*-*p*-menth-2-ene has a much lower optical activity (McNiven and Read, previous paper; Hückel and Wagner, *Ber.*, 1941, **74**, 657), this terpene must be (−)-*trans*-*p*-menth-2-ene.

Thus it has been shown that (−)-carvomenthone is configurationally related to (−)-*trans*-*p*-menth-2-ene. It can therefore be concluded that (−)-carvomenthone has the *trans*-configuration. In addition it has already been shown by Read and Hendry (*loc. cit.*) that (−)-menthone is configurationally related to (+)-*trans*-*p*-menth-2-ene. Accordingly, (−)-menthone and its related compounds must belong to the same steric series as (+)-carvomenthone, thus confirming Hückel's conclusion (*J. pr. Chem.*, 1941, **158**, 21).

Since quaternary ammonium hydroxides on thermal decomposition form olefins by a bimolecular ionic mechanism dependent on *trans*-elimination (Dhar, Hughes, Ingold, Mandour, Maw, and Woolf, *J.*, 1948, 2117), the exclusive formation of (−)-*trans*-*p*-menth-2-ene from carvomenthyltrimethylammonium hydroxide can occur only if the configuration of the groups on carbon atom 2 is as shown in (III):



(III)



(IV)

It follows that (+)-carvomenthylamine must have a similar configuration.

Consequent on the establishment of the configuration of this amine, which was obtained by sodium reduction of the oxime of (−)-carvomenthone, the configuration of (−)-*neo*carvomenthylamine obtained by catalytic hydrogenation of the oxime of (−)-carvomenthone (Hückel and Wilip, *loc. cit.*) must be the epimeric form shown in (IV) with the amino-group on carbon atom 2 in the *cis*-position to the methyl group.

Experimental

(Analyses are by Drs. Weiler and Strauss, Oxford. M. p.s are uncorrected.)

Carvomenthyltrimethylammonium Hydroxide.—(+)-Carvone (240 g.), $[\alpha]_D^{15}$ +53·60° (*l* = 1 dm.; homogeneous) in methanol (500 ml.) was hydrogenated, 2 teaspoonsfuls of Raney nickel catalyst being used. After 3 hours at room temperature the absorption of hydrogen stopped when one molecular equivalent had been added. After the temperature had been raised to 90° the second molecular equivalent was taken up in a further 3½ hours. The resulting (−)-carvomenthone (221 g., 90%), $[\alpha]_D^{16}$ −24·29° (*l* = 1 dm.; homogeneous) was brought to the equilibrium mixture (Read and Johnston, *J.*, 1934, 230) by allowing it to stand at room temperature for 19 hours in sodium ethoxide solution (42 g. of sodium in 1 l. of ethanol). The resulting carvomenthone mixture, $[\alpha]_D^{16}$ −13·41° (*l* = 1 dm.; homogeneous), and hydroxylamine acetate in ethanol at room temperature (24 hours) gave crystalline (−)-carvomenthone oxime (130 g., 62·5%), m. p. 97—98°, $[\alpha]_D^{16}$ −39·3° (*c*, 1·27 in ethanol). This oxime (67 g.) was reduced by sodium and ethanol, Wallach's procedure (*Annalen*, 1893, **276**, 301) being used, to carvomenthylamine (58 g., 94%). The hydrochloride after 4 recrystallisations from ethyl acetate had the constant rotation $[\alpha]_D^{17}$ +11·0° (*c*, 2·2 in water). The benzoyl derivative had m. p. 165°, $[\alpha]_D^{15}$ +41·0° (*c*, 1·17 in chloroform). Read and Johnston (*loc. cit.*) reported $[\alpha]_D^{16}$ +12·2° for the hydrochloride, and m. p. 161°, $[\alpha]_D^{25}$ +45·1° for the benzoyl derivative. The free carvomenthylamine

liberated from the above hydrochloride (5·3 g.) was converted into (+)-carvomenthyltrimethylammonium iodide (7·1 g., 95%) by the method used for (−)-menthylamine by McNiven and Read (foregoing paper); after recrystallising from acetone this gave colourless crystals of *(+)-carvomenthyltrimethylammonium iodide*, m. p. 141°, $[\alpha]_D^{15}$ +24·8° (*c*, 2·18 in water) (Found: I, 39·9. $C_{13}H_{26}NI$ requires I, 39·1%). The iodide was converted into the quaternary ammonium hydroxide by the method described by McNiven and Read (*loc. cit.*) for (−)-menthylamine.

Pyrolysis of Carvomenthyltrimethylammonium Hydroxide.—The above quaternary ammonium hydroxide from 4·7 g. of iodide, after concentration on a steam-bath, was distilled at 166°/20 mm. (bath-temp.). The oily distillate, after being washed with dilute hydrochloric acid and then sodium carbonate solution and dried (Na_2SO_4), gave (−)-*trans-p*-menth-2-ene (0·05 g., 3%), $[\alpha]_D^{15}$ −139·5° (*c*, 1·86 in ethanol). An oily base (2·1 g., 80%), probably carvomenthyldimethylamine, was recovered from the acid washings.

Attempted Racemisation of (−)-trans-p-*Menth*-2-*ene.*—This menthene was refluxed with alcoholic toluene-*p*-sulphonic acid for 4 hours, following Hückel's method (*Annalen*, 1940, **543**, 191). Since the resulting menthene had $[\alpha]_D^{15}$ −142·2° (*c*, 1·10 in ethanol), no racemisation had taken place.

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36. *Steroids and Related Compounds. Part XIII.* The Preparation of 21-Hydroxypregna-4 : 17-dien-3-one.*

By D. K. PATEL, V. PETROW, R. ROYER, and (MRS.) I. A. STUART-WEBB.

Novel methods for the conversion of 3β-acetoxyandrost-5-en-17-one into pregna-5 : 17-diene-3β : 21-diol (I) and 21-hydroxypregna-4 : 17-dien-3-one (VIII) are described. Attempts to hydroxylate an ethyl 20-cyanopregn-17-en-21-oate (V) in order to obtain an ethyl 17α-hydroxy-20-ketopregnan-21-oate are reported

IN the course of studies (Magrath, Morris, Petrow, and Royer, *J.*, 1950, 2393) on the preparation of "Compound S" from 3β-acetoxyandrost-5-en-17-one, the latter was condensed with ethoxyethynylmagnesium bromide, and the resulting 3β-acetoxy-17α-ethoxyacetylenylandrost-5-en-17β-ol treated with ethanolic sulphuric acid. Reduction of the resultant ethyl 3β-acetoxypregna-5 : 17-dien-21-oate (II; R = Ac) with lithium aluminium hydride furnished pregna-5 : 17-diene-3β : 21-diol (I) (cf. Heusser, Eichenberger, and Plattner, *Helv. Chim. Acta*, 1950, **33**, 370, 1088), which was regarded as a key intermediate in this series of reactions. From it, by partial acetylation and oxidation, we hoped to obtain 21-hydroxypregna-4 : 17-dien-3-one (VIII), the facile conversion of which into "Compound S" has already been described by Miescher and Schmidlin (*ibid.*, p. 1840).

In contrast to results obtained with such compounds as 3α : 21-dihydroxypregn-17-en-11-one (Sarett, *J. Biol. Chem.*, 1946, **162**, 601), preferential acylation of the $C_{(21)}$-hydroxyl group of (I) proved only partly successful. Careful treatment with acetic anhydride or *p*-nitrobenzoyl chloride in pyridine gave only the diacyl derivatives admixed with unchanged material. Triphenylmethyl chloride in pyridine surprisingly failed to react. Succinic anhydride in pyridine gave an unsatisfactory yield of a product to which the structure 3β-hydroxypregna-5 : 17-diene-21-yl hydrogen succinate has been assigned

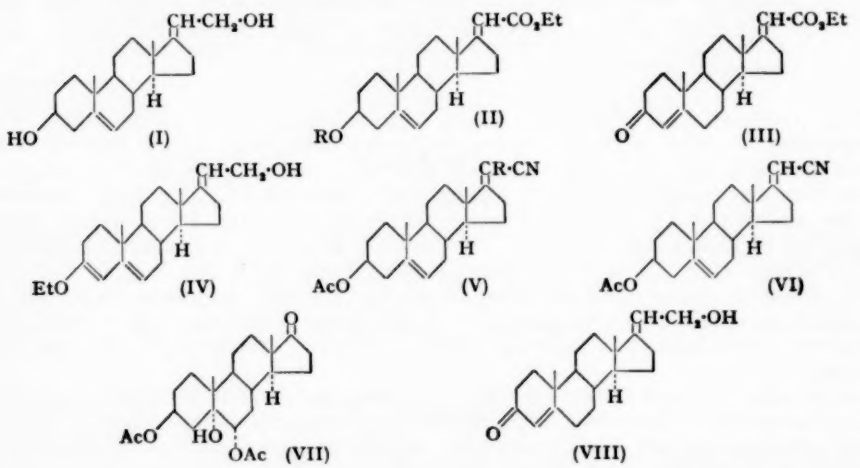
* Part XII, *J.*, 1951, 901.

(cf. Sarett, *loc. cit.*). Attempts to oxidise this compound by the Oppenauer method, however, proved unsuccessful, no doubt owing to its sparing solubility in appropriate solvents.

We, therefore, turned our attention to an alternative route to (VIII), employing ethyl 3β-hydroxypregna-5 : 17-dien-21-oate (II; R = H) as a starting material. Oppenauer oxidation of this compound proceeded with great facility to furnish ethyl 3-ketopregna-4 : 17-dien-21-oate (III) in excellent yield. Protection of the 3-keto-group in this compound before reduction of the carbethoxy-group was effected by conversion into the enol ether (see Meystre and Miescher, *Helv. Chim. Acta*, 1949, **32**, 1758) from which, by reaction with lithium aluminium hydride, 3-ethoxypregna-3 : 5 : 17-trien-21-ol (IV) was readily obtained in 90% yield. However, the final step in this series of reactions, the regeneration of the 3-keto-group, could not be effected by the usual procedure employing methanolic hydrochloric acid. In place of (VIII), an αβ-unsaturated ketone containing one methoxyl group was obtained, to which the formulation 21-methoxypregna-4 : 17-dien-3-one has been assigned. Conversion of (IV) into (VIII) was ultimately achieved by simply dissolving the enol ether in ordinary chloroform, which evidently contained sufficient acid to catalyse the required hydrolysis.

An alternative approach to pregna-5 : 17-diene-3β : 21-diol (I) consisted of condensation of 3β-acetoxyandrost-5-en-17-one with compounds of the cyanoacetic ester type. Initial experiments revealed that the reaction could not be effected by means of catalysts such as piperidine acetate, acetic anhydride, or zinc chloride or by the procedure employed by Smith and Webb (*J.*, 1948, 1358) in the bile acid series. However, refluxing 3β-acetoxyandrost-5-en-17-one with ethyl cyanoacetate in benzene in the presence of a little acetic acid, with regular additions of ammonium acetate (cf. Cragoe, Robb, and Sprague, *J. Org. Chem.*, 1950, **15**, 381) readily gave ethyl 3β-acetoxy-20-cyanopregna-5 : 17-dien-21-oate (V; R = CO_2Et) in nearly quantitative yield. This structure was confirmed by (i) careful hydrolysis followed by Oppenauer oxidation, to give ethyl 20-cyano-3-ketopregna-4 : 17-dien-21-oate, and (ii) hydrolysis to give 20-cyano-3β-hydroxypregna-5 : 17-dien-21-oic acid, characterised by conversion into a highly crystalline methyl ester.

Hydroxylation of (V; R = CO_2Et) with osmium tetroxide was accompanied by fission * of the $C_{(17)}$–$C_{(20)}$ bond with formation, after acetylation, of 3β : 6α-diacetoxy-5α-



hydroxyandrostan-17-one (VII). Its behaviour on treatment with this reagent thus stands in marked contrast to that established by Sarett (*loc. cit.*) for the related 20-cyanopregn-17-en-21-ols, where formation of the ketol side chain typical of cortisone occurs.

* Possibly by the sodium sulphite used in working-up.

Similar results were obtained employing ethyl 3β-acetoxy-20-cyano*allo*pregn-17-en-21-oate (from 3β-acetoxyandrostan-17-one and ethyl cyanoacetate), hydroxylation of which likewise led to removal of the pregnane side chain with formation of 3β-acetoxyandrostan-17-one.

Decarboxylation of 20-cyano-3β-hydroxypregna-5 : 17-dien-21-oic acid invariably gave a complex mixture of products. By distillation at 140—160°/10^{-3} mm., however, followed by fractional crystallisation and acetylation, 3β-acetoxy-20-cyanopregna-5 : 17-diene (VI) could be obtained in *ca.* 30% yield; however, the cyano-group proved resistant to ethanolic sulphuric acid. Reduction with lithium aluminium hydride, followed by treatment with nitrous acid and acetylation, gave 3β : 21-diacetoxypregna-5 : 17-diene (cf. I).

Condensation of 3β-acetoxyandrost-5-en-17-one with malononitrile furnished 3β-acetoxy-20 : 20-dicyanopregna-5 : 17-diene (V; R = CN) in 95% yield. The cyano-groups present in this compound likewise (cf. VI above) proved resistant to hydrolysis.

By reaction of 3β-acetoxyandrost-5-en-17-one with ethyl cyanothiolacetate, ethyl 3β-acetoxy-20-cyanopregna-5 : 17-diene-20-thiolcarboxylate (V; R = CO·SEt) was readily obtained, but this compound could not be converted into the corresponding 3β-acetoxy-20-cyanopregna-5 : 17-dien-21-ol owing to concomitant reduction of the cyano-group.

Experimental

M. p.s are uncorrected. Ultra-violet absorption spectra were determined in alcoholic solution by Dr. R. E. Stuckey and Mr. P. Stross, B.Sc., Analytical Department, The British Drug Houses, Ltd.

3β : 21-*Di*-p-*nitrobenzoyloxypregna*-5 : 17-*diene*, prepared by treating pregna-5 : 17-diene-3β : 21-diol (1 g.) in pyridine (20 ml.) with *p*-nitrobenzoyl chloride (1·05 mols.) for 24 hours at room temperature, formed crystals (from benzene), m. p. 206—207° (Found : C, 68·5; H, 5·9; N, 4·6. $C_{35}H_{38}O_8N_2$ requires C, 68·4; H, 6·2; N, 4·6%). Unchanged material was recovered from the mother-liquors.

3β-*Hydroxypregna*-5 : 17-*diene*-21-*yl hydrogen succinate*, prepared by treating (I) (4·3 g.) for 2 hours at 28—29° with pyridine (22 ml.) saturated with succinic anhydride, formed crystals, m. p. 210—211°, $[\alpha]_D^{22}$ −48° ± 2° (*c*, 1·024 in dioxan), from ethanol (Found : C, 71·0; H, 8·8. $C_{25}H_{36}O_5$ requires C, 72·1; H, 8·7%).

Ethyl 3-*Ketopregna*-4 : 17-*dien*-21-*oate* (III).—Ethyl 3β-hydroxypregna-5 : 17-dien-21-oate (10 g.) in *cyclo*hexanone (66 ml.) was heated under reflux for 1 hour with a solution of aluminium *iso*propoxide (11 g.) in toluene (44 ml.). The mixture was then poured into water and steam-distilled for 4 hours. The non-volatile fraction was extracted with chloroform, and the product purified by passage in benzene through a column of alumina (activated; *ex* B.D.H.; 250 g. in a column 30 mm. wide), which was developed with benzene–ether. Evaporation of the bulked benzene and benzene–ether eluates left *ethyl* 3-*ketopregna*-4 : 17-*dien*-21-*oate* (70%), m. p. 181° (from methanol), $[\alpha]_D^{22}$ +88° (*c*, 1·044 in chloroform), $\lambda_{max.}$ 230 mμ (log ε = 4·4) (Found : C, 77·7; H, 9·2. $C_{23}H_{32}O_3$ requires C, 77·5; H, 9·0%).

Ethyl 3β-*Ethoxypregna*-3 : 5 : 17-*trien*-21-*oate*.—A mixture of the foregoing ester (3·0 g.), toluene-*p*-sulphonic acid (300 mg.) and ethyl orthoformate (30 ml.) was heated under reflux for 1 hour, whereafter the mixture was evaporated under reduced pressure. The partly crystalline residue was covered with methanol, one drop of pyridine added, and the mixture left overnight, after which the separated solids were collected, washed carefully with the minimum quantity of ice-cold methanol, and purified by crystallisation from methanol–ether. *Ethyl* 3β-*ethoxypregna*-3 : 5 : 17-*trien*-21-*oate* (81%) has m. p. 129—130°, $[\alpha]_D^{22}$ −177° (*c*, 0·580 in pyridine), $\lambda_{max.}$ 232 mμ (log ε = 4·5) (Found : C, 77·8; H, 9·3. $C_{25}H_{36}O_3$ requires C, 78·1; H, 9·4%).

3β-*Ethoxypregna*-3 : 5 : 17-*trien*-21-*ol* (IV).—A solution of the foregoing compound (1·6 g.) in dry ether (25 ml.) was added dropwise with stirring during 15 minutes to lithium aluminium hydride (800 mg.) in ether (100 ml.), and the mixture refluxed for 30 minutes. The product (91%), isolated in the usual way, was purified from methanol–ether, to give 3β-*ethoxypregna*-3 : 5 : 17-*trien*-21-*ol*, m. p. 118—119°, $[\alpha]_D^{22}$ −153° (*c*, 0·444 in pyridine), $\lambda_{max.}$ 241 mμ (log ε = 4·3) (Found : C, 80·2; H, 10·0. $C_{23}H_{34}O_2$ requires C, 80·7; H, 9·7%).

21-*Hydroxypregna*-4 : 17-*dien*-3-*one* (VIII).—A solution of the foregoing enol ether (250 mg.) in " reagent-grade " chloroform (50 ml.) was kept at room temperature for 72 hours. After evaporation of the solvent the pale yellow oil was treated with hexane containing a trace of

acetone, to give 21-hydroxypregna-4 : 17-dien-3-one (63%), needles, m. p. 137°, $[\alpha]_D^{24}$ +112° (*c*, 0·838 in ethanol) (Found : C, 79·9; H, 9·5. Calc. for $C_{21}H_{30}O_2$: C, 80·3; H, 9·6%). Ruzicka and Muller (*Helv. Chim. Acta*, 1939, **22**, 416) give m. p. 138—139° (corr.), $[\alpha]_D$ +116° ± 2° (in ethanol).

21-*Methoxypregna*-4 : 17-*dien*-3-*one*.—A solution of 3β-ethoxypregna-3 : 5 : 17-trien-21-ol (500 mg.) in methanol (30 ml.) was heated under reflux with 2N-hydrochloric acid for 1 hour. Dilution with water, followed by extraction with ether, afforded an oil which was dissolved in benzene and chromatographed on alumina (9 g. in a column of 12 mm. diameter). The benzene and benzene–ether eluates yielded 21-*methoxypregna*-4 : 17-*dien*-3-*one* (27%), m. p. 106—107° (from hexane), $[\alpha]_D^{24}$ +103° (*c*, 0·428 in ethanol) (Found : C, 79·6; H, 9·8; OMe, 11·1. $C_{22}H_{32}O_2$ requires C, 80·5; H, 9·8; OMe, 9·5%). The presence of an αβ-unsaturated ketonic residue in the compound was indicated by the formation of a scarlet dinitrophenylhydrazone.

Ethyl 3β-*Acetoxy*-20-*cyanopregna*-5 : 17-*dien*-21-*oate* (V; R = CO_2Et).—3β-Acetoxyandrost-5-en-17-one (6·6 g.) and ethyl cyanoacetate (4·5 g.) in benzene (80 ml.) and pure acetic acid (19 ml.) were heated under reflux in a flask fitted with a Dean and Stark separator, ammonium acetate (7 g.) being added every 3 hours during a total of 30 hours. The cooled reaction mixture was poured on ice, and the product isolated with ether. Crystallisation from ethanol afforded *ethyl* 3β-*acetoxy*-20-*cyanopregna*-5 : 17-*dien*-21-*oate* (90—95%), m. p. 182—184°, $[\alpha]_D^{27}$ −39·4° (*c*, 0·674 in chloroform), $\lambda_{max.}$ 238 mμ (log ε = 4·1) (Found : C, 73·5; H, 8·0; N, 3·4. $C_{26}H_{35}O_4N$ requires C, 73·4; H, 8·2; N, 3·3%).

20-*Cyano*-3β-*hydroxypregna*-5 : 17-*dien*-21-*oic Acid*.—To a solution of the foregoing ester (1·0 g.) in methanol (15 ml.), potassium carbonate (600 mg.) in water (5 ml.) was added and the mixture heated under reflux for 30 minutes. Addition of dilute sulphuric acid (to pH 2—3) gave 20-*cyano*-3β-*hydroxypregna*-5 : 17-*dien*-21-*oic acid* (80%), m. p. 253—254° (decomp.) (from acetone) (Found : C, 73·9; H, 8·2. $C_{22}H_{29}O_3N$ requires C, 74·3; H, 8·2%).

The *methyl* ester, crystallised from ether–light petroleum, m. p. 190—192°, $[\alpha]_D^{20}$ −44·8° (*c*, 1·084 in chloroform) (Found : C, 74·5; H, 8·3. $C_{23}H_{31}O_3N$ requires C, 74·8; H, 8·5%), was obtained by treating a suspension of the acid in dry ether with excess of diazomethane.

Ethyl 20-*Cyano*-3-*ketopregna*-4 : 17-*dien*-21-*oate*.—The ester (V; R = CO_2Et) (6·4 g.) dissolved in absolute ethanol (10 ml.) containing sulphuric acid (0·75 ml.; *d* 1·84) was heated under reflux for 2 hours, and then the mixture was poured into ice-water. The neutral fraction, isolated in the usual way, gave the 3-hydroxy-ester (85—90%), m. p. 151—153°, from aqueous ethanol. This compound (1·0 g.) was mixed with a solution (9 ml.; 16%) of aluminium *tert.*-butoxide in benzene, and the mixture evaporated to dryness. The residue in dry toluene (7 ml.) and *cyclo*hexanone (6 ml.) was heated under reflux for 2 hours and the product isolated in the usual way. *Ethyl* 20-*cyano*-3-*ketopregna*-4 : 17-*dien*-21-*oate* (85%) [from ether–light petroleum (b. p. 40—60°)] had m. p. 132—134°, $[\alpha]_D^{22}$ +143·2° (*c*, 1·980 in chloroform) (Found : C, 75·7; H, 8·3; N, 3·7. $C_{24}H_{31}O_3N$ requires C, 75·6; H, 8·2; N, 3·7%).

3β : 6α-*Diacetoxy*-5α-*hydroxyandrostan*-17-*one* (VII).—To ethyl 3β-acetoxy-20-cyanopregna-5 : 17-dien-21-oate (2·0 g.) in dry ether (50 ml.) was added a solution of osmium tetroxide (2·0 g.) in dry ether (20 ml.), followed by pyridine (0·2 ml.). After being kept overnight at room temperature, the mixture was taken to dryness and the residue heated under reflux for 3 hours with sodium sulphite (10·5 g.) in water (24 ml.) and ethanol (35 ml.). The hot mixture was filtered and the residue extracted with successive 100-ml. portions of 60, 70, 80, and 90%, and absolute ethanol. The bulked extracts were taken to dryness and the residue dissolved in water (50 ml.) and repeatedly extracted with chloroform (6 × 30 ml.). The combined extracts were washed until neutral and the solvent removed. Acetylation of the residue gave 3β : 6α-diacetoxy-5α-hydroxyandrostan-17-one (550 mg.), needles, m. p. 242—245°, $[\alpha]_D^{24}$ +57·7° (*c*, 1·952 in chloroform) (Found : C, 68·1; H, 8·3. Calc. for $C_{23}H_{34}O_6$: C, 68·0; H, 8·4%). Ehrenstein and Decker (*J. Org. Chem.*, 1940, **5**, 544) give m. p. 253—254°, $[\alpha]_D$ +63·6° (in acetone).

Ethyl 3β-*acetoxy*-20-*cyano*allo*pregn*-17-*en*-21-*oate*, prepared (90%) from 3β-acetoxyandrostan-17-one and ethyl cyanoacetate by the procedure described for (V; R = CO_2Et) and crystallised from ethanol, had m. p. 164—166°, $[\alpha]_D^{20}$ +30·1° (*c*, 2·832 in chloroform) (Found : C, 73·1; H, 8·4; N, 3·6. $C_{26}H_{37}O_4N$ requires C, 73·1; H, 8·4; N, 3·3%).

Hydroxylation with osmium tetroxide gave 3β-acetoxyandrostan-17-one, m. p. 115—117°, not depressed on admixture with an authentic specimen. Reduction of the latter compound with lithium aluminium hydride, followed by acetylation, furnished 3β : 17β-diacetoxyandrostane, m. p. 125—127°, $[\alpha]_D$ −2·0° (*c*, 1·76 in acetone). Shoppee (*Helv. Chim. Acta*, 1940, **23**, 740) gives m. p. 127—129°, $[\alpha]_D$ −1·0° ± 1° (in acetone).

3β-*Acetoxy*-20-*cyanopregna*-5 : 17-*diene* (VI).—20-Cyano-3β-hydroxypregna-5 : 17-dien-21-oic acid (2·0 g.) was distilled at 140—160°/10^{-3} mm. and the glassy product crystallised by addition of light petroleum. Purification from ether–light petroleum gave the hydroxy-nitrile, m. p. 144—146°, in *ca.* 30% yield, acetylation of which furnished 3β-*acetoxy*-20-*cyanopregna*-5 : 17-*diene* (95%), m. p. 176—178°, $[\alpha]_D^{22}$ −71·5° (*c*, 2·466 in chloroform) (Found : C, 78·0; H, 8·8; N, 4·1. $C_{23}H_{31}O_2N$ requires C, 78·1; H, 8·9; N, 4·0%).

3β : 21-*Diacetoxypregna*-5 : 17-*diene*.—To the foregoing compound (1·7 g.) in dry ether (100 ml.) was added a solution of lithium aluminium hydride (1·5 g.) in ether (100 ml.), and the mixture heated under reflux for 2 hours. The product formed a viscous liquid which gave a positive test for a primary aliphatic amine. It (1·0 g.) was dissolved in acetic acid (2 ml.) and water (0·4 ml.) and the ice-cooled solution was treated with sodium nitrite (1·0 g.) in the minimum quantity of ice-water, after which the mixture was allowed to warm to room temperature. Further quantities of acetic acid (2 ml.) and sodium nitrite (500 mg.) were then added, after which the mixture was heated for 1 hour on the steam-bath, cooled, and poured into water. Ether-extraction gave a product which, after acetylation overnight with acetic anhydride (1 ml.) in pyridine (1 ml.), was purified by chromatography (30 g. alumina) in 95% light petroleum–benzene, to give 3β : 21-*diacetoxypregna*-5 : 17-*diene* (300 mg.), needles (from methanol), m. p. 135—136°, $[\alpha]_D^{24}$ −52·7° (*c*, 0·902 in chloroform) (Found : C, 75·2; H, 9·3. Calc. for $C_{25}H_{36}O_4$: C, 75·0; H, 9·0%), not depressed on admixture with authentic material (Heusser, Eichenberger, and Plattner, *loc. cit.*).

3β-*Acetoxy*-20 : 20-*dicyanopregna*-5 : 17-*diene* (V; R = CN), prepared from 3β-acetoxyandrost-5-en-17-one (3·3 g.) and malononitrile (1·0 g.), formed prisms (from ethanol), m. p. 184—186°, $[\alpha]_D^{20}$ −56·2° (*c*, 1·880 in chloroform) (Found : C, 76·1; H, 8·1; N, 7·5. $C_{24}H_{30}O_2N_2$ requires C, 76·0; H, 8·3; N, 7·4%).

Ethyl 3β-*Acetoxy*-20-*cyanopregna*-5 : 17-*diene*-20-*thiolcarboxylate* (V; R = CO·SEt).—Recrystallised cyanoacetic acid (17 g.) was dissolved in dry ether (75 ml.) and the cooled solution treated with phosphorus pentachloride (40 g.) in portions during 20 minutes. After removal of ether and phosphorus halides under reduced pressure, cyanoacetyl chloride was obtained, b. p. 58°/0·1 mm. (cf. Schroeter, *J. pr. Chem.*, 1922, **105**, 165). It was treated, in dry ether (50 ml.), with the lead salt of ethanethiol (17 g.), in portions, during 15 minutes. Lead halide was removed and washed with ether, and the filtrate and washings were taken to dryness and distilled, to give *ethyl cyanothiolacetate* (7 g.), b. p. 126°/10 mm. (Found : C, 46·3; H, 5·5; N, 10·7; S, 24·7. C_5H_7ONS requires C, 46·5; H, 5·4; N, 10·9; S, 24·8%).

Condensation of this ester with 3β-acetoxyandrost-5-en-17-one gave *ethyl* 3β-*acetoxy*-20-*cyanopregna*-5 : 17-*diene*-20-*thiolcarboxylate*, large prisms (95%) (from chloroform–*n*-hexane), m. p. 216—218°, $[\alpha]_D^{21}$ −40·6° (*c*, 1·364 in chloroform) (Found : C, 70·7; H, 7·6; N, 3·2; S, 6·8. $C_{26}H_{35}O_3NS$ requires C, 70·7; H, 8·0; N, 3·2; S, 7·2%).

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37. *Potential Thiophen Chemotherapeutics. Part II.* A Thiophen Analogue of "Marfanil" (Maphenide).*

By J. CYMERMAN and D. FAIERS.

2-Aminomethylthiophen-5(?)-sulphonamide hydrochloride has been obtained from 2-acetamidomethyl- and from 2-phthalimidomethyl-thiophen.

THE antibacterial properties of *p*-substituted benzenesulphonamides are well known, in particular, that of "Marfanil" (maphenide), *p*-sulphamylbenzylamine (Klarer, *Klin. Wschr.*, 1941, **20**, 1250; Domagk, *ibid.*, 1942, **21**, 448; Jensen and Schmith, *Z. Immunitatsf.*, 1942,

* The communication, "Some 2-Thienyl Alkyl Sulphones" (*J.*, 1949, 1666), is regarded as Part I.

102, 261). It has been shown in numerous instances that replacement of a benzene nucleus by the 2-thienyl group leads to isosteres possessing comparable biological activity, and the preparation of a thiophen analogue of maphenide has now been carried out by two parallel routes.

Acetylation of 2-aminomethylthiophen (Hartough, *J. Amer. Chem. Soc.*, 1948, **70**, 4018) afforded a product which was separated by fractional distillation into crystalline 2-acetamidomethyl- and oily 2-diacetylaminomethyl-thiophen. This recalls the similar behaviour of benzylamine on acetylation (Holmes and Ingold, *J.*, 1925, 1820). Chlorosulphonation of 2-acetamidomethylthiophen afforded what is presumed to be the 5-sulphonyl chloride, which gave the desired 2-acetamidomethylthiophen-5-sulphonamide by reaction with either alcoholic ammonia or, in better yield, dry ammonium carbonate. Acid hydrolysis of this sulphonamide gave a good yield of 2-aminomethylthiophen-5-sulphonamide hydrochloride, m. p. 234—235°. Chlorosulphonation is presumed to have occurred in the 5-position (cf. Cundiff and Estes, *J. Amer. Chem. Soc.*, 1950, **72**, 1424; Steinkopf and Kohler, *Annalen*, 1937, **532**, 264).

Work directed towards a rigid proof of the orientation of the sulphonyl group is in progress.

In the other preparation, reaction of 2-chloromethylthiophen (Blicke and Burckhalter, *J. Amer. Chem. Soc.*, 1942, **64**, 478) with potassium phthalimide by Ing and Manske's method (*J.*, 1926, 2348) gave 2-phthalimidomethylthiophen, previously prepared by Hartough (*J. Amer. Chem. Soc.*, 1948, **70**, 1146) from 2-aminomethylthiophen and phthalic anhydride. Chlorosulphonation of 2-phthalimidomethylthiophen readily afforded the (presumably) 5-sulphonyl chloride in good yield. The halogen atom in this substance possessed remarkably low reactivity towards ammonia but reaction with ammonium carbonate at 180° gave 61% of the sulphonamide. Hydrolysis of this by means of hydrazine hydrate (Ing and Manske, *loc. cit.*) readily afforded the desired salt (m. p. 234—235°), identical with the sample obtained by the previous route. The free base was obtained as a very hygroscopic solid, m. p. 96—97°.

Maphenide hydrochloride has been reported (Heideman and Rutledge, *J. Pharm. Exp. Therap.*, 1948, **93**, 451) to possess an ultra-violet absorption spectrum showing a single maximum at 2650 Å. The ultra-violet absorption spectrum of a 95%-alcoholic solution of the hydrochloride of our thiophen analogue, obtained by using a Beckman quartz spectrophotometer, model DU, showed a single maximum at 2440 Å, $\varepsilon = 10\,950$.

Professor S. D. Rubbo has kindly examined this compound against a range of organisms and reports that it is devoid of appreciable bacteriological activity.

Experimental

2-*Acetamidomethylthiophen*.—A mixture of acetic acid (120 g., 2 mols.), acetic anhydride (25·2 g., 0·2 mol.) and 2-aminomethylthiophen (22·6 g., 0·2 mol.; b. p. 45—52°/0·5 mm.) was refluxed for 2·5 hours. Most of the acetic acid was removed by distillation and the residue poured on ice. The oil which separated was extracted with ether and distillation of the washed (sodium hydrogen carbonate) and dried (Na_2SO_4) extracts afforded a colourless oil (28 g., 90%), b. p. 144—152°/1 mm., which partly solidified to give white crystals (15 g.) separating from ether–light petroleum (b. p. 40—70°) as needles, m. p. 44—45° (Found: N, 8·8; S, 20·35. C_7H_9ONS requires N, 9·0; S, 20·65%), of 2-*acetamidomethylthiophen*.

The liquid portion of the distillates from several runs (63 g.) gave, on fractionation, 2 main fractions: (*a*) b. p. 125—130°/1·5 mm. (20·5 g.), n_D^{15} 1·5514, of 2-*diacetylaminomethylthiophen* (Found: C, 55·2; H, 6·0; N, 7·25. $C_9H_{11}O_2NS$ requires C, 54·8; H, 5·6; N, 7·1%), sparingly soluble in cold N-hydrochloric acid; and (*b*) b. p. 136—143°/1·5 mm. (31·5 g.), solidifying to crystals, m. p. 44—45°, of the monoacetyl derivative, easily soluble in cold N-hydrochloric acid.

Heating a mixture of the amine (1 mol.) and acetic anhydride (1 mol.) with acetic acid (0·5 or 5 mols.) gave yields of 66 and 80% of the acetyl derivatives respectively.

2-*Acetamidomethylthiophen-5-sulphonyl Chloride*.—Crystalline 2-acetamidomethylthiophen (15·5 g., 0·1 mol.) was added during 30 minutes to well-stirred chlorosulphonic acid (redistilled; 70 g., 0·6 mol.), at <5° (ice). The mixture was stirred at 25° for 30 minutes and finally at 50°

for 30 minutes. The oil was poured on ice (200 g.), and the viscous oil which separated was washed well with ice-water. Trituration with dry ether gave a solid (9 g., 37%) which crystallised from benzene in prisms, m. p. 88—89°, of *2-acetamidomethylthiophen-5-sulphonyl chloride* (Found: N, 5·45; S, 25·25. $C_7H_8O_3NS_2Cl$ requires N, 5·5; S, 25·25%).

2-Acetamidomethylthiophen-5-sulphonamide.—(*a*) The above sulphonyl chloride (1·2 g., 0·005 mol.) was added gradually to well-stirred ethanol (100 c.c.) saturated with ammonia (16% w/v) at 0°. The solid dissolved slowly, and the solution was then set aside for 30 minutes. The residue left by evaporation of the solution *in vacuo* was dissolved in sodium hydroxide solution (10%), then filtered, and the filtrate acidified with hydrochloric acid. Concentration of the solution to crystallisation gave plates, m. p. 123—124° (0·2 g., 17%), of *2-acetamidomethylthiophen-5-sulphonamide* (Found: C, 36·5; H, 4·45; N, 11·4; S, 27·6. $C_7H_{10}O_3N_2S_2$ requires C, 35·9; H, 4·3; N, 12·0; S, 27·4%).

(*b*) A well-stirred mixture of the sulphonyl chloride (2·5 g., 0·01 mol.) and dry ammonium carbonate (10 g., 0·1 mol.; "AnalaR") was heated at 120° (oil-bath) for 30 minutes. After the addition of ice-water, the solid was filtered off and recrystallised from alcohol or hot water, giving 1·5 g. (64%) of plates, m. p. 123—124°, undepressed on admixture with the material obtained by method (*a*) (Found: C, 36·15; H, 4·3; N, 12·1; S, 27·5%).

2-Aminomethylthiophen-5-sulphonamide.—(i) A mixture of 2-acetamidomethylthiophen-5-sulphonamide (5 g.) and hydrochloric acid (3N; 50 c.c.) was refluxed for 8 hours and then concentrated *in vacuo* to crystallisation, giving white crystals of *2-aminomethylthiophen-5-sulphonamide hydrochloride* (3·5 g., 73%), crystallising from concentrated hydrochloric acid in plates, m. p. 234—235° (decomp.) (Found: C, 26·65; H, 4·0; S, 27·5. $C_5H_9O_2N_2S_2Cl$ requires C, 26·25; H, 4·0; S, 28·0%). Light absorption: $\lambda_{max.}$ 2440 Å, $\varepsilon = 10{,}950$, in 95% ethanol.

2-Phthalimidomethylthiophen.—To a solution of 2-chloromethylthiophen (freshly-distilled; b. p. 45—50°/0·5 mm.; 20 g., 0·15 mol.) in xylene (100 c.c.) was added a mixture of dry phthalimide (33 g., 0·22 mol.) and anhydrous potassium carbonate (15·5 g., 0·11 mol.). The reaction mixture was refluxed vigorously for 4 hours, then cooled and washed with water and sodium hydroxide solution (2N). The xylene solution was dried ($CaCl_2$) and the solvent removed *in vacuo*, leaving a residue (20 g., 55%), m. p. 123—124°, which on crystallisation from alcohol or chloroform–light petroleum (b. p. 40—70°) afforded needles, m. p. 127—127·5°, of 2-phthalimidomethylthiophen (Found: C, 63·85; H, 3·75; N, 5·8. Calc. for $C_{13}H_9O_2NS$: C, 64·15; H, 3·7; N, 5·75%) (Hartough, *J. Amer. Chem. Soc.*, 1948, **70**, 1146, gives m. p. 126—127°).

2-Phthalimidomethylthiophen-5-sulphonyl Chloride.—2-Phthalimidomethylthiophen (7·2 g., 0·03 mol.) was added gradually during 20 minutes to well-stirred chlorosulphonic acid (21 g., 0·18 mol.) at <5° (ice). Use of the reaction conditions described above for 2-acetamidomethylthiophen gave 7·5 g. (76%) of *2-phthalimidomethylthiophen-5-sulphonyl chloride*, m. p. 138—147°, crystallising from chloroform–light petroleum (b. p. 40—70°) in prismatic needles, m. p. 147—147·5° (Found: C, 45·2; H, 2·3; N, 4·15; Cl, 10·5. $C_{13}H_8O_4NSCl$ requires C, 45·65; H, 2·35; N, 4·1; Cl, 10·4%). The substance was unchanged after crystallisation from alcohol.

2-Phthalimidomethylthiophen-5-sulphonamide.—An intimate mixture of the sulphonyl chloride (3 g., 0·009 mol.) and ammonium carbonate (19 g., 0·2 mol.) was slowly heated to 180° and kept at this temperature for 30 minutes. The cooled mixture was washed with ice-water and the residue crystallised from ethyl acetate, affording prisms of *2-phthalimidomethylthiophen-5-sulphonamide* (1·7 g., 61%), m. p. 171—173° (Found: N, 8·75. $C_{13}H_{10}O_4N_2S_2$ requires N, 8·7%). The sulphonyl chloride was unchanged after treatment with aqueous ammonia (*d* 0·88), alcoholic ammonia, ammonia in boiling benzene, or ammonium carbonate at 170° for 10 minutes, while reaction at 180° for 10 minutes gave the sulphonamide in 13·5% yield (m. p. 169—173°).

2-Aminomethylthiophen-5-sulphonamide.—(ii) 2-Phthalimidomethylthiophen-5-sulphonamide (0·5 g., 0·0015 mol.) was heated on the steam-bath with absolute alcohol (8 c.c.), and the mixture treated with hydrazine hydrate (0·3 c.c., 50% w/w), giving a clear solution immediately. After a few minutes, the intermediate compound was precipitated. The mixture was heated for 30 minutes and then treated with hydrochloric acid (1 c.c.; 10N) to decompose the intermediate. After 5 minutes, the phthalhydrazide was filtered off and the alcoholic filtrate evaporated *in vacuo*, leaving the hydrochloride (0·17 g., 50%) as white plates, m. p. 234—235° (decomp.), undepressed on admixture with the sample prepared by method (i).

A solution of the hydrochloride in the minimum amount of water was basified to pH 9 with 0·1N-sodium hydroxide, the solution evaporated *in vacuo*, and the residue extracted with

absolute alcohol. Addition of ether to the alcoholic extract gave 2-aminomethylthiophen-5-sulphonamide as a white, extremely hygroscopic, micro-crystalline solid, m. p. 96—97°, for which satisfactory analytical values could not be obtained (Found: N, 11·6. Calc. for $C_5H_8O_2N_2S_3$: N, 14·55%).

The authors acknowledge their indebtedness to Professor S. D. Rubbo for kindly undertaking the bacteriological tests, and to Mrs. E. Bielski for microanalyses.

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38. *Infra-red Spectra of Derivatives of Pyrimidine.*

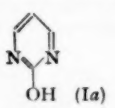
By L. N. SHORT and H. W. THOMPSON.

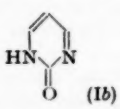
The vibrational spectra between 2 and 25μ of a large number of simple derivatives of pyrimidine have been measured. These include many hydroxy- and amino-derivatives with varying numbers and types of substituent. The spectra have been compared empirically, and partial interpretations have been made on the basis of what is already known about the vibrational frequencies of such key groups as OH, C=O, NH, etc. Particular consideration has been given to the question of keto–enol or lactim–lactam tautomerism which may occur in many of these compounds, and although there are many ambiguities the balance of evidence seems in most cases to favour a ketonic structure for the hydroxy-derivatives, and the presence of amino- rather than imino-forms. The spectra provide reference data which may be useful for analysis and identification.

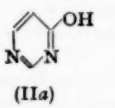
THE role of derivatives of pyrimidine in the chemistry of nucleic acids justifies an examination of any method likely to aid their qualitative or quantitative determination or the identification of particular compounds. Spectroscopic methods are obviously relevant, and some ultra-violet absorption measurements have been reported. The greater specificity of infra-red vibrational spectra in relation to nuclear configuration might in principle make these spectra even more useful. There are two limitations, namely, that infra-red measurements with aqueous solutions are effectively impossible, and that the vibrational spectra of such complex compounds may prove too complex for detailed analysis.

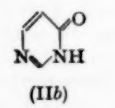
However, the vibrational spectra of the solid materials may provide some evidence about outstanding structural problems. One of these concerns the tautomerism which is possible with hydroxy- and amino-derivatives and which may affect their function in metabolism. This tautomerism between ketonic–enolic (lactam–lactim) forms or amino–imino-structures may be influenced by other nuclear substituents. The problem has been studied by several methods with rather indefinite results.

2-Hydroxy- and 4-hydroxy-pyrimidine may have the extreme tautomeric forms (I*a*), (I*b*), and (II*a*), (II*b*), to which will be added various polar structures which may combine


(I*a*)


(I*b*)

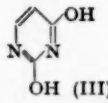

(II*a*)

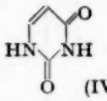

(II*b*)

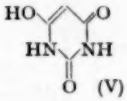
in the final resonance hybrid. From chemical evidence, Arndt (*Rev. Fac. Sci. Istanbul*, 1944, *A*, **2**, 19) concluded that when it is compatible with the maintenance of aromatic character in the ring, the hydroxypyrimidines will have the lactam form. On this basis uracil (2 : 4-dihydroxypyrimidine) (III) will have the structure (IV), and barbituric acid (2 : 4 : 6-trihydroxypyrimidine) will be represented by (V).

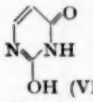
From ultra-violet measurements on ethanol solutions of a series of derivatives, Austin (*J. Amer. Chem. Soc.*, 1934, **56**, 2141) concluded that uracil has structure (VI), but

Loufbourow, Stimson, and Hart (*ibid.*, 1943, **65**, 148) decided from similar measurements in water at different hydrogen-ion concentrations that it is doubly ketonic (IV), a result

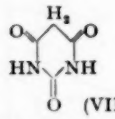

(III)

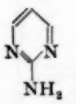

(IV)

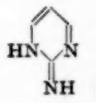

(V)

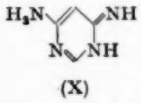

(VI)

confirmed by Marshall and Walker (*J.*, 1951, 1004). The formula (V) for barbituric acid retains some degree of aromatic character, whereas (VII) does not, and 5 : 5-diethylbarbituric acid shows no selective ultra-violet absorption such as would indicate the latter structure.


(VII)


(VIII)


(IX)

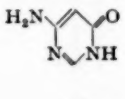

(X)

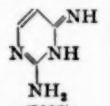
Similarly, 2-aminopyrimidine (VIII) could exist in the imino-form (IX) with which polar structures might participate in the final hybrid, and correspondingly with 4-aminopyrimidine. An amino-group in the 5-position would not be capable of tautomerism.

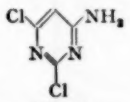
Cavalieri and Bendich (*J. Amer. Chem. Soc.*, 1950, **72**, 2587) have recently argued from some ultra-violet results that 4 : 6-diamino-, 4-hydroxy-2-amino- and 2 : 4-diaminopyrimidine have the structures (X), (XI), and (XII).

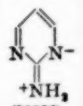
As regards physical properties, the high melting points and low solubilities of the hydroxy-derivatives in non-polar media indicate the participation of polar structures. which may be less marked with the amino-derivatives.

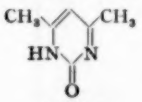
Clews and Cochran (*Acta Cryst.*, 1948, **1**, 4; 1949, **2**, 46) have recently suggested from *X*-ray work that 4-amino-2 : 6-dichloropyrimidine exists in the amino-form (XIII). In this and other compounds (2-amino-4 : 6-dichloro-, 2-amino-4-chloro-6-methyl-, and 4 : 6-diamino-5-bromo-pyrimidine) however a shortening of the extranuclear carbon–nitrogen bond may be explained in terms of an imino-structure or a considerable contribution to the resonance hybrid of the polar forms, such as (XIV). Pitt (*Acta Cryst.*,


(XI)


(XII)


(XIII)


(XIV)


(XV)

1948, **1**, 168), also from *X*-ray data, found that in 2-hydroxy-4 : 6-dimethylpyrimidine, the very short carbon–oxygen distance is compatible with a ketonic form (XV).

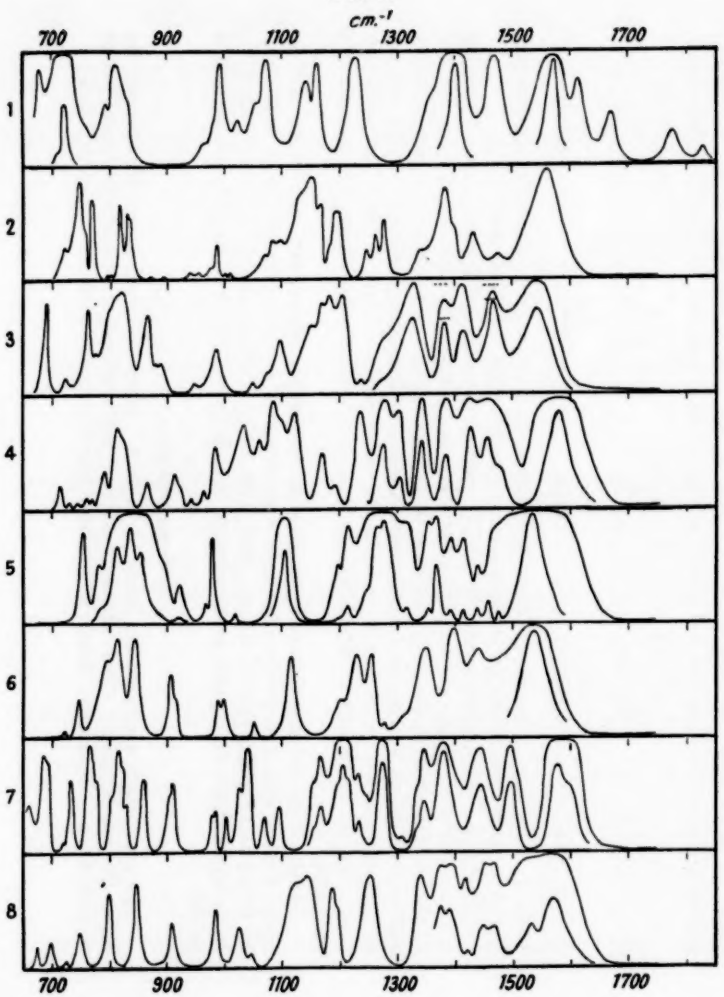
Since the vibration frequencies of the C=O and N–H bonds in different types of grouping are now fairly well known, it seemed appropriate to examine the infra-red spectra of some of the simpler derivatives of pyrimidine from this aspect. We have to expect complex and individualistic spectra as a whole, with other complications caused by varying degrees of hydrogen-bridge formation in the different compounds. There is the added difficulty that only the solids can be examined, so that comparisons with dilute solutions cannot be made.

Similar infra-red measurements on some of the simpler pyrimidines and several nucleic acids, nucleotides, and nucleosides have been reported by Brownlie (*J.*, 1950, 3062) and by Blout and Fields (*J. Biol. Chem.*, 1949, **178**, 335; *J. Amer. Chem. Soc.*, 1950, **72**, 479). In so far as they overlap our own in regard to results or interpretation they are discussed below.

Experimental

The compounds examined are listed in Table 1. Many were tested for homogeneity by paper chromatography. When sufficient material was available it was recrystallised, but in no case was any significant change of spectrum found after this process.

Fig. 1.


cm.⁻¹
700
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1100
1300
1500
1700
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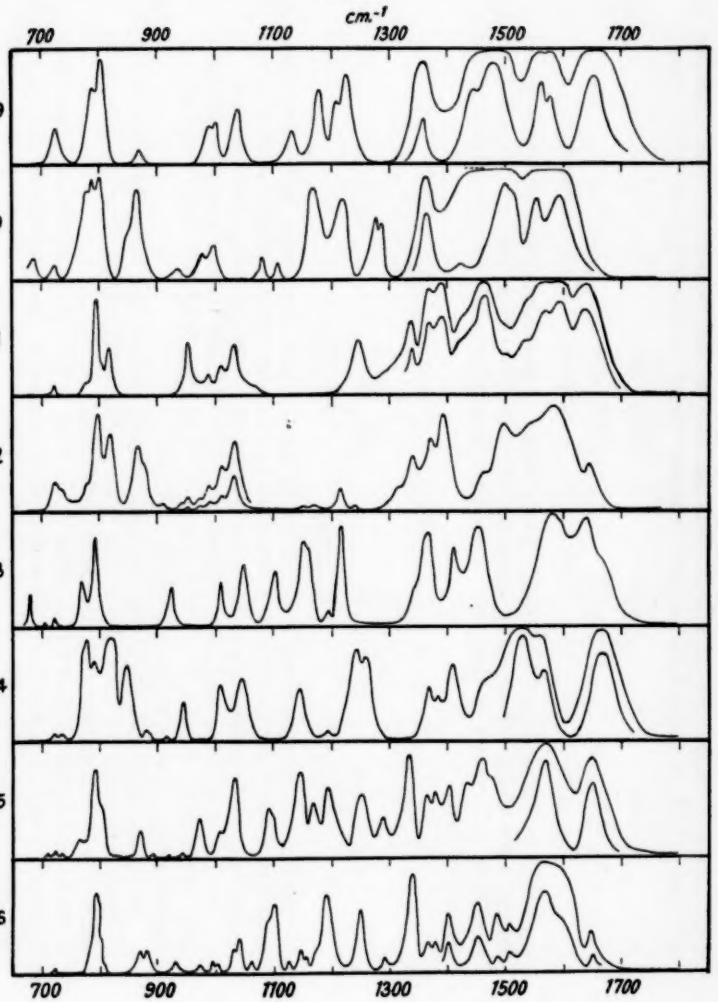
2
3
4
5
6
7
8

Fig. 2.


cm.⁻¹
700
900
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1300
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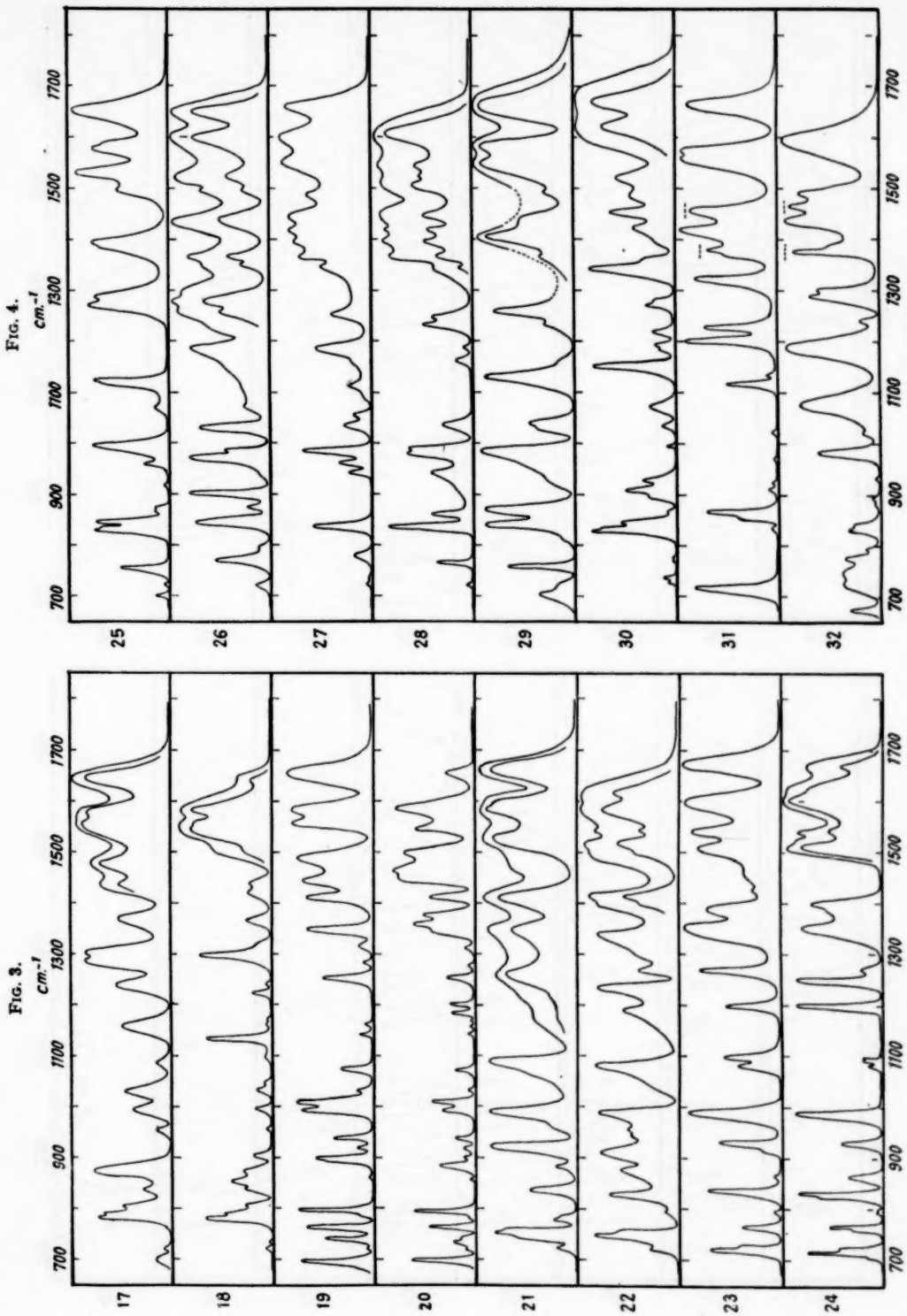
16


700
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cm.⁻¹
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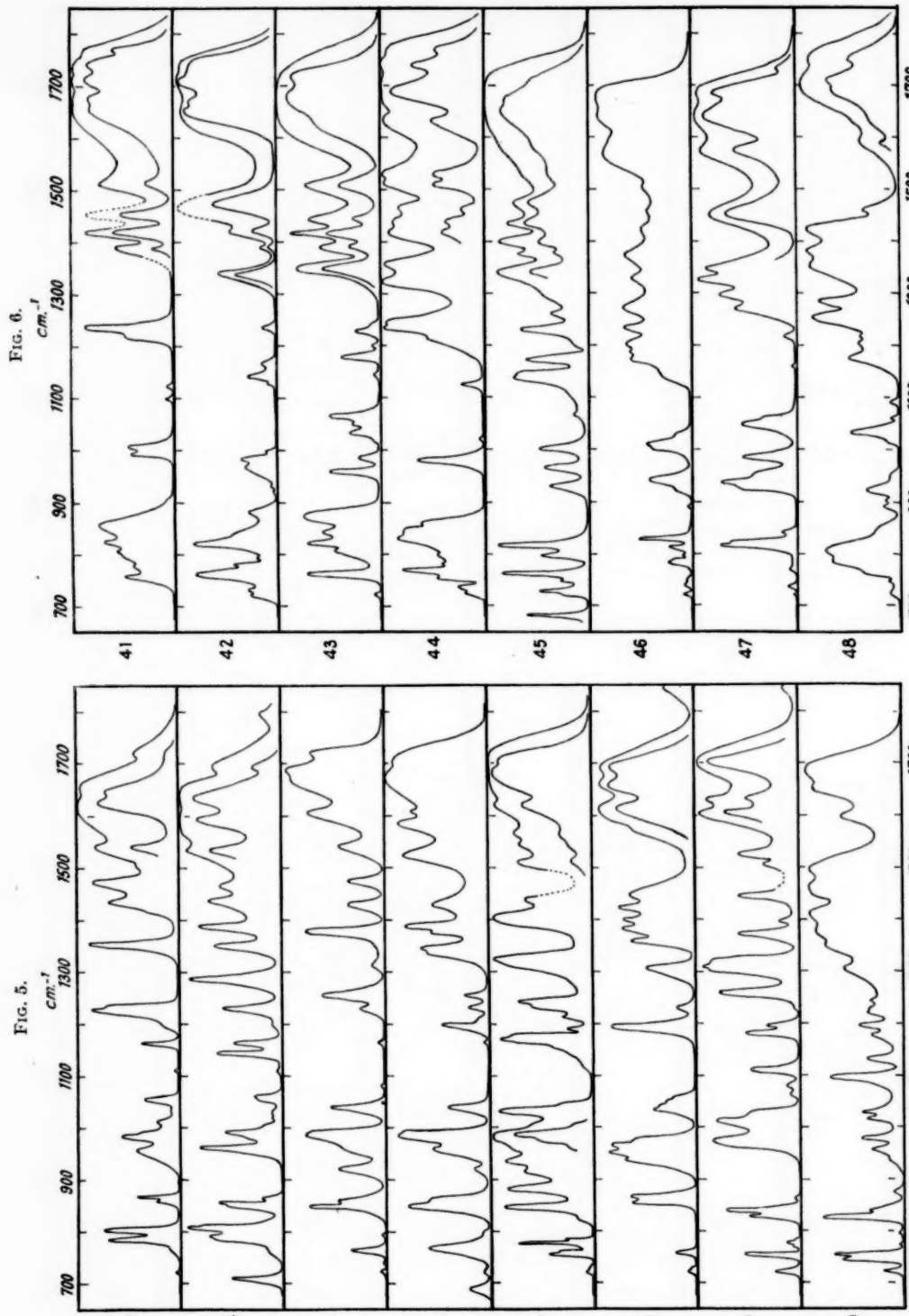
32

FIG. 3.

FIG. 4.


33
34
35
36
37
38
39
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41
42
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44
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46
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48
700
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1300
1500
1700
cm.⁻¹

FIG. 5.

FIG. 6.

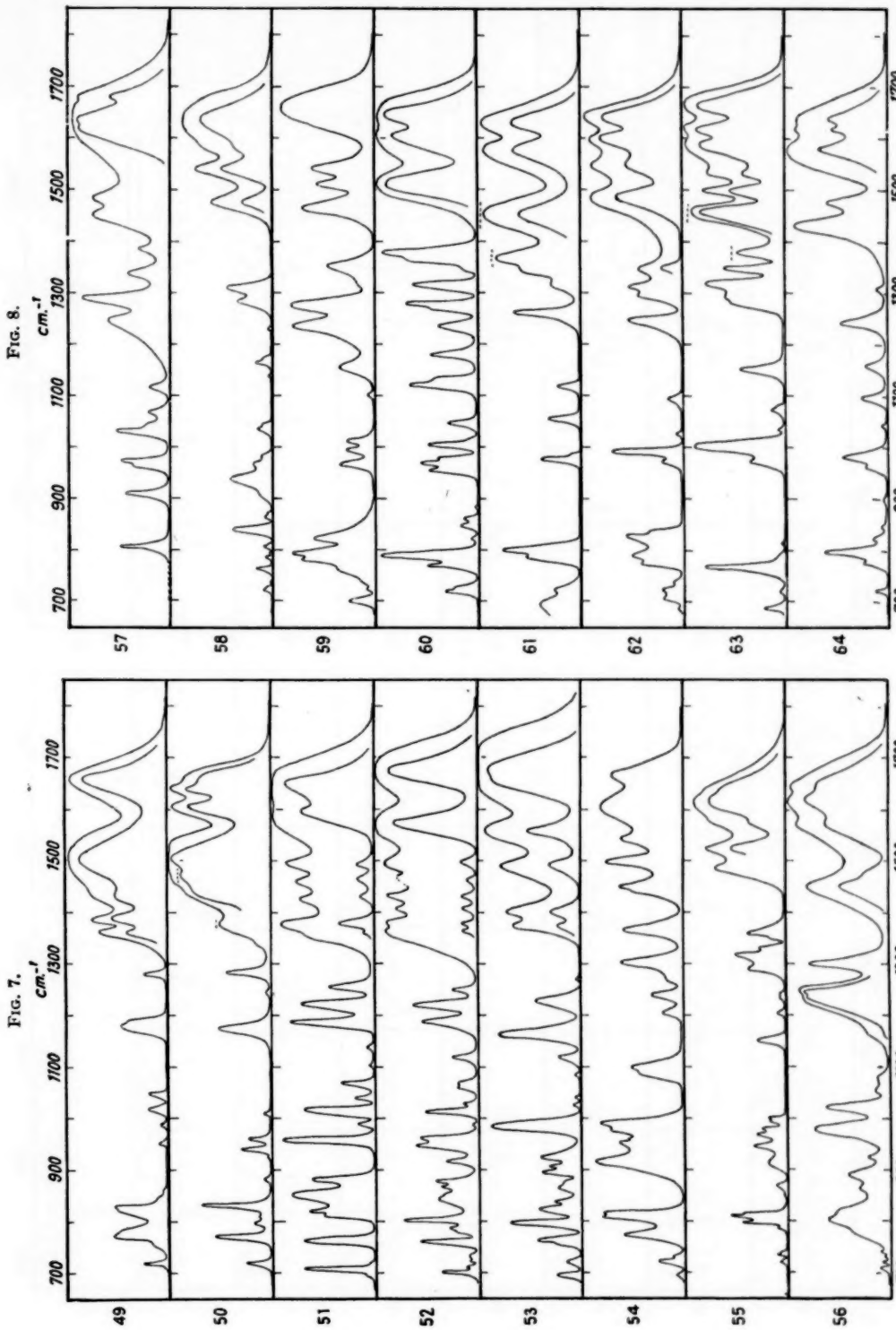

700
900
1100
1300
1500
1700
cm.⁻¹
49
50
51
52
53
54
55
56

FIG. 7.

700
900
1100
1300
1500
1700
cm.⁻¹
57
58
59
60
61
62
63
64

FIG. 8.

FIG. 9.

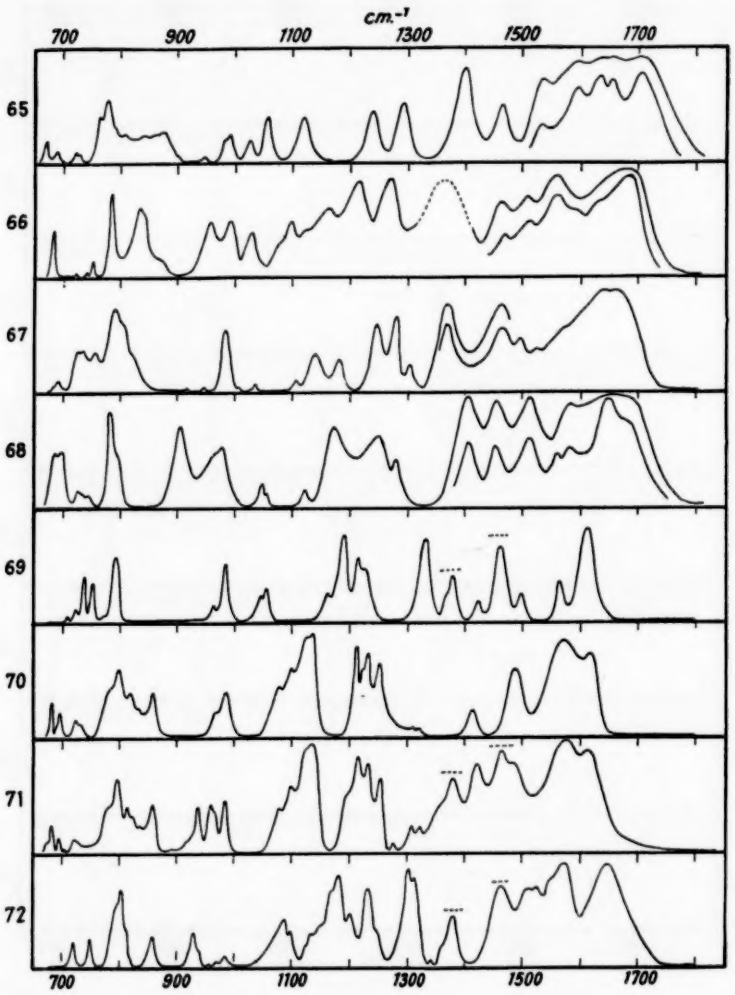
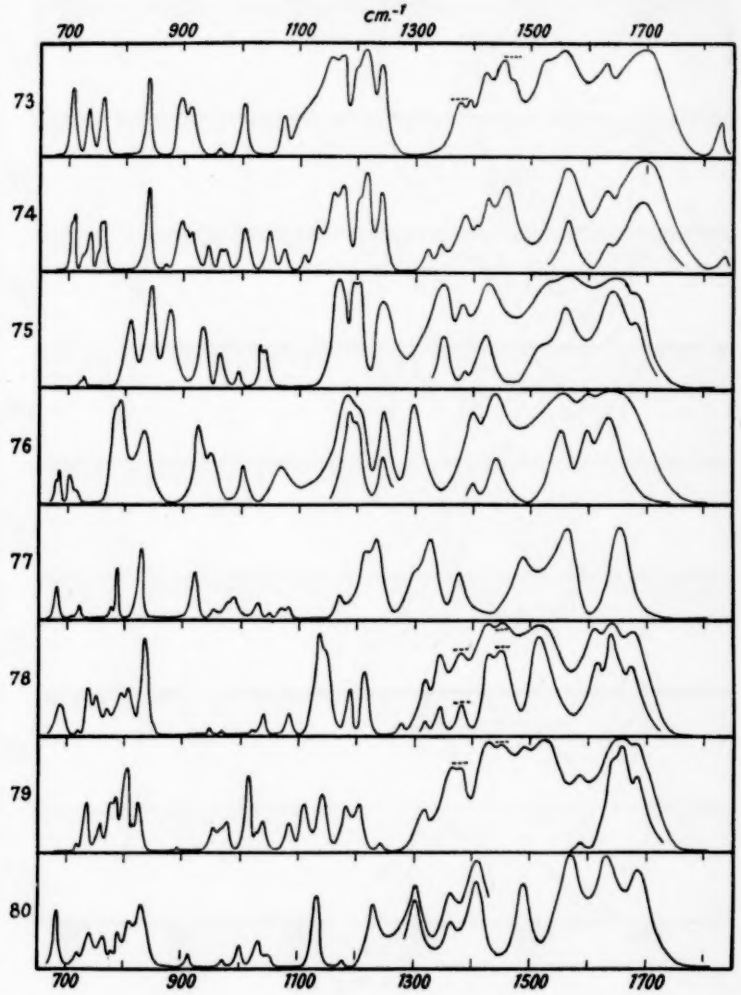

cm.⁻¹
700
900
1100
1300
1500
1700
65
66
67
68
69
70
71
72

FIG. 10.


cm.⁻¹
700
900
1100
1300
1500
1700
73
74
75
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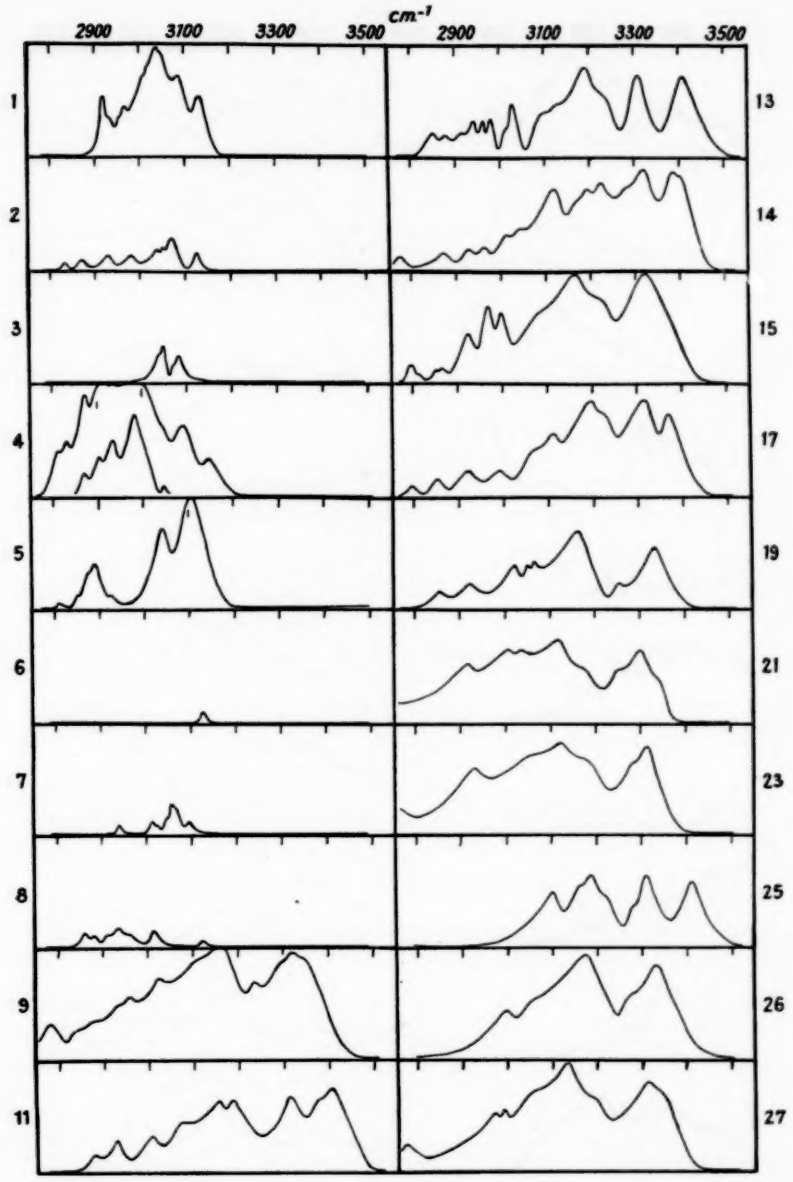
77
78
79
80

FIG. 11.


cm⁻¹
2900
3100
3300
3500
1
2
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11
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19
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26
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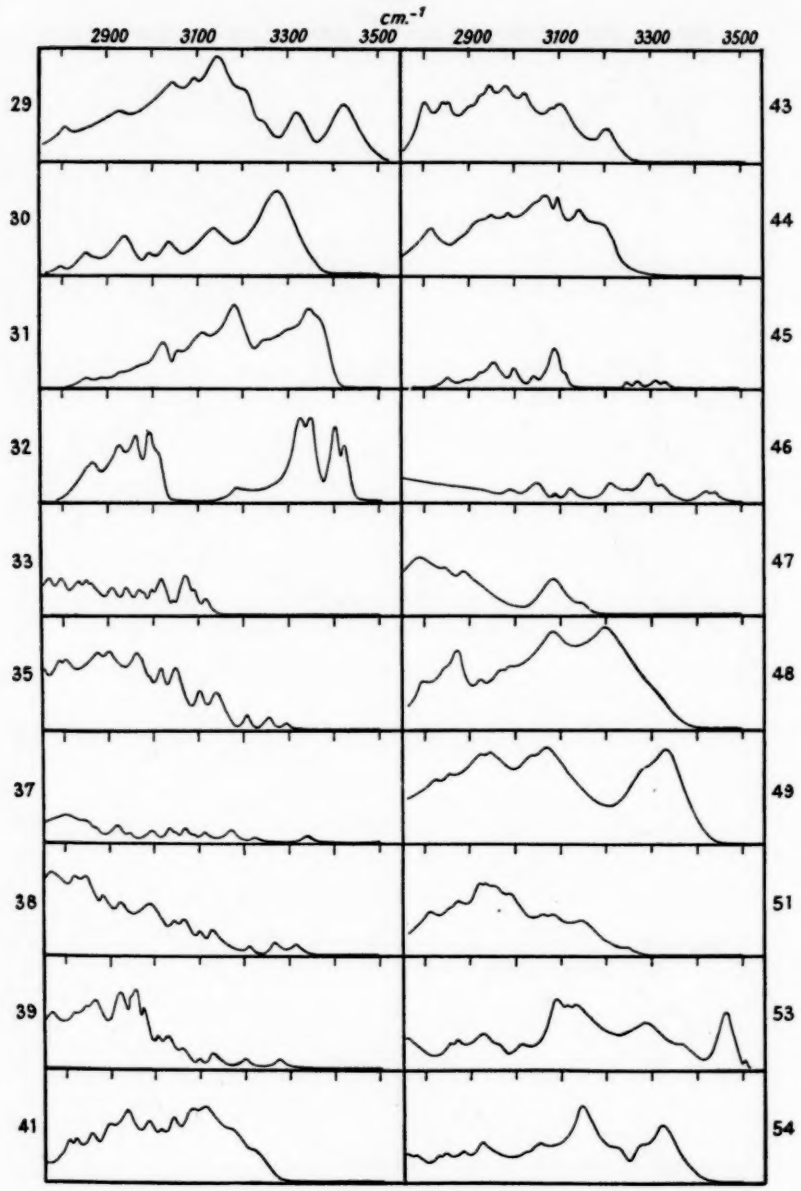
FIG. 12.


cm.⁻¹
2900
3100
3300
3500
2900
3100
3300
3500
29
30
31
32
33
35
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43
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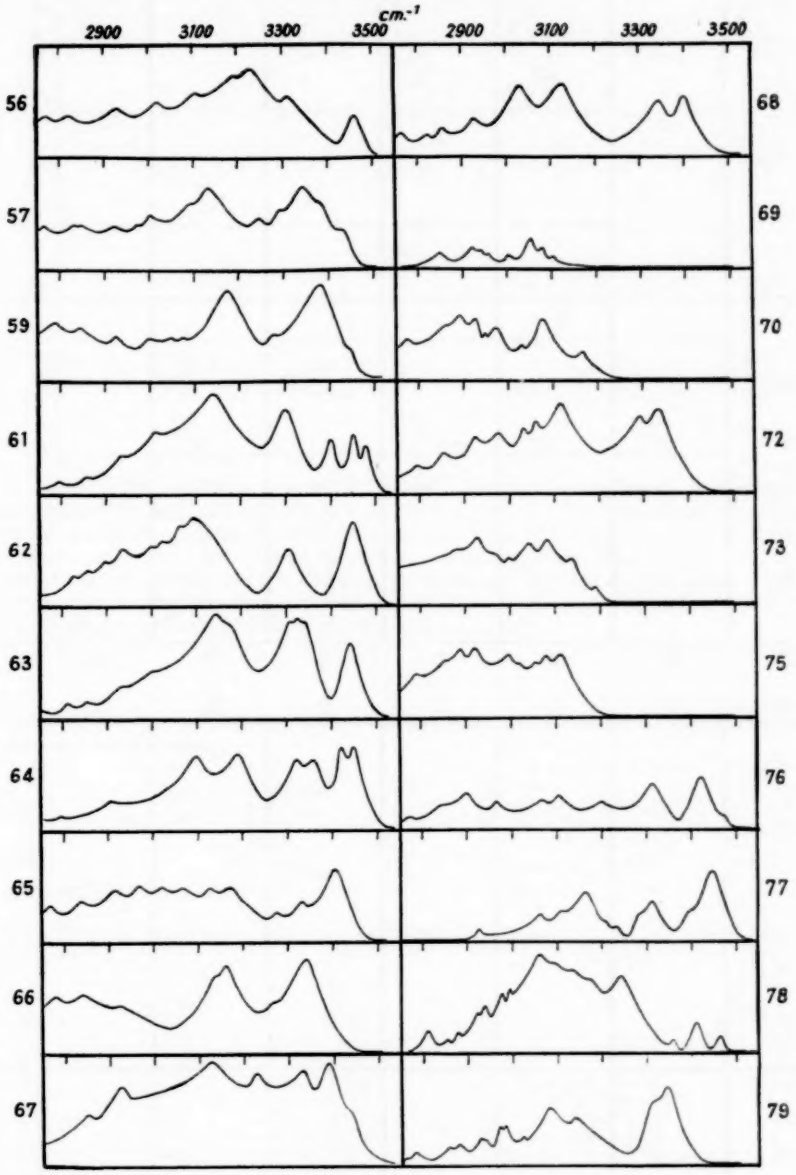
54

FIG. 13.


cm.⁻¹
2900
3100
3300
3500
56
57
59
61
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72
73
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78
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FIG. 14.

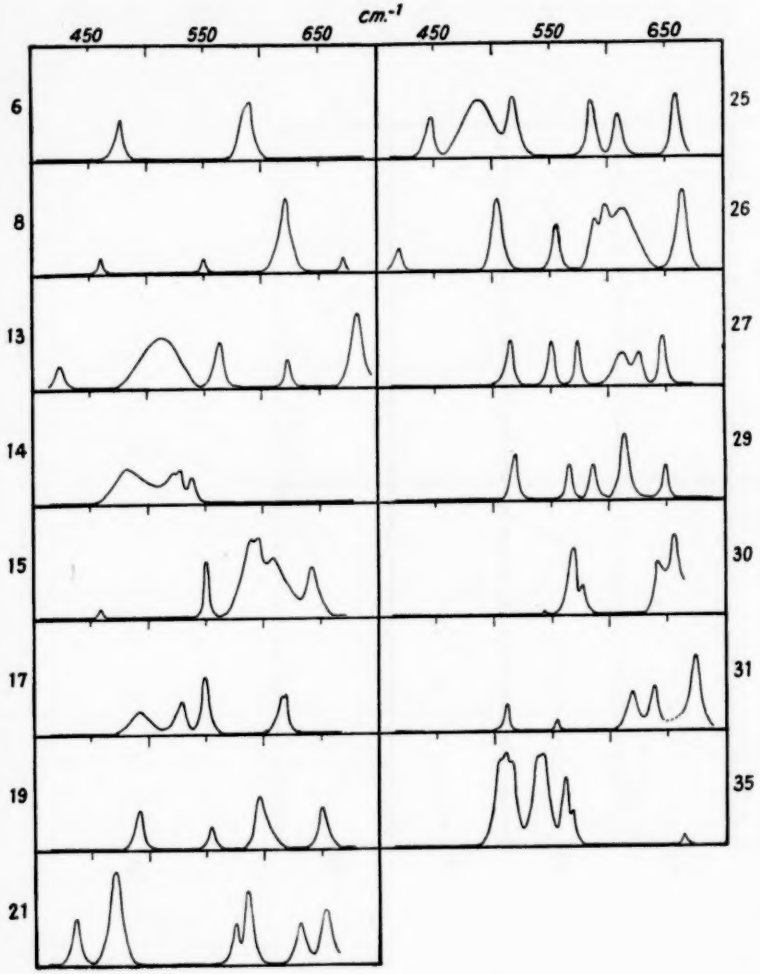
cm.-1
450
550
650
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FIG. 15.

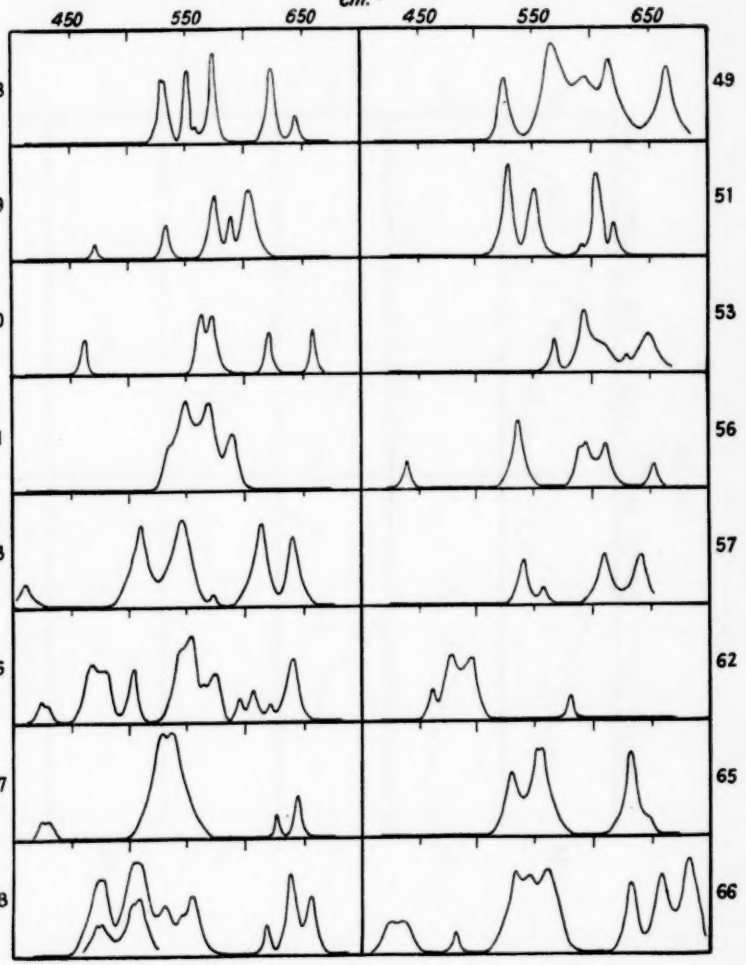
cm.-1
450
550
650
38
39
40
41
43
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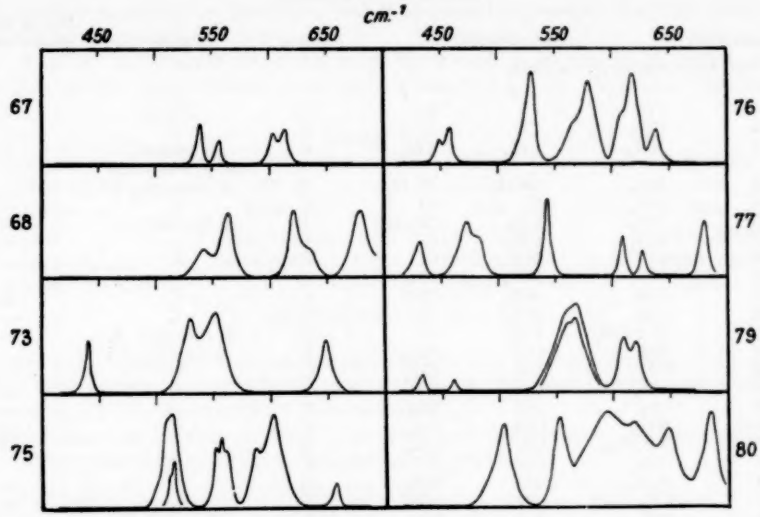
FIG. 16.



The spectra were determined with a Perkin Elmer 12C instrument, prisms of lithium fluoride, sodium chloride, and potassium bromide being used. The compounds were examined as solids suspended in paraffin or perfluorokerosene; in a few cases there was a slight doubt about bands in the narrow region near 7μ where paraffin has strong bands.

Some of the compounds were deuterated by treatment with deuterium oxide. Whilst the extent to which deuteration had occurred was in some cases doubtful, the removal of bands at 3μ and appearance of others near 4μ was a useful indication.

TABLE 1.

No. of spectrum	Substituent(s)		Source [a]	Purity [b]	No. of spectrum of deutero-cpd.
(a) *Monosubstituted pyrimidines*					
2	2-Cl		D	m. p. 63·5—64°	—
9	2-NH_2		A	C	10
31	5-NH_2		G	m. p. 173—174°	—
33	2-OH		E	C	34
35	4-OH		A	R (toluene),	36
69	2-SH		D	m. p. 230°	—
(b) 2 : 4-*Disubstituted pyrimidines*					
	2	4			
3	Cl	Cl	A	R (ether), m. p. 62°	—
4	OC_2H_5	OC_2H_5	C	—	—
7	OC_6H_5	OC_6H_5	E	C	—
23	Cl	NH_2	B	C	24
41	OH	OH	A	C	42
59	OH	NH_2	E	C	60
61	NH_2	NH_2	A	R (toluene), m. p. 143—144°	—
70	SH	SH	E	Anal. pure	71
72	SH	NH_2	E	Anal. pure	—
73	SH	OH	C	I	74
(c) 4 : 6-*Disubstituted pyrimidines*					
	4	6			
21	NH_2	Cl	C	C	22
37	OH	CH_3	C	C	—
46	OH	OH	C	R (water), S	—
54	OH	NH_2	E	C	55
62	NH_2	NH_2	C	C	—

TABLE 1—*continued.*

No. of spectrum	Substituents			Source [a]	Purity [b]	No. of spectrum of deutero-cpd.
(d) 2 : 4 : 6-*Trisubstituted pyrimidines*						
	2	4	6			
5	Cl	Cl	Cl	C	Liquid	—
6	CH_3	Cl	Cl	C	C	—
11	NH_2	CH_3	CH_3	C	R (water), m. p. 149—150°	12
13	NH_2	OCH_3	OCH_3	B	R (toluene), S	—
14	NH_2	Cl	Cl	C	C	—
15	NH_2	CH_3	OC_2H_5	B	R (petrol.), S	16
17	NH_2	CH_3	Cl	C	C	18
25	Cl	NH_2	Cl	C	C	—
26	Cl	NH_2	CH_3	B	C	—
27	CH_3	NH_2	CH_3	C	C	28
29	CH_3	NH_2	Cl	C, B	C	—
30	NH·CH_3	CH_3	Cl	B	R (toluene), S	—
38	CH_3	OH	CH_3	C	R (water), S	—
43	OH	OH	CH_3	C	C	—
47	CH_3	OH	OH	C	C	—
48	OH	OH	OH	C	I	—
49	NH_2	OH	CH_3	C	C	50
51	$N(CH_3)_2$	OH	CH_3	B	C	52
53	NH_2	OH	Cl	C	C	—
56	SCH_3	OH	NH_2	B	C	—
57	CH_3	OH	NH_2	C	C	58
64	NH_2	NH_2	NH_2	C	R (alcohol)	—
65	OH	OH	NH_2	C	C	—
66	NH_2	OH	OH	C	C	—
67	NH_2	NH_2	OH	C	I	—
68	OH	NH_2	NH_2	C	C	—
75	SH	OH	CH_3	B	C	—
76	SH	OH	NH_2	B	R (alcohol), S	—
77	NH_2	CH_3	CN	B	R (alcohol), S	—
80	NH_2	CH_3	CO·NH_2	B	C	
(e) *Miscellaneous derivatives*						
1	none			C	—	—
19	4-NH_2, 5-C_6H_5			C	C	20
44	2 : 4-di-OH, 5-NO_2			A	C	—
63	4 : 6-di-NH_2, 5-Br			C	—	—
8	2-CH_3, 4 : 6-di-O·CH_3, 5-NO_2			B	C	—
32	2-CH_3, 4 : 6-di-O·CH_3, 5-NH_2			B	C	—
39	2-S·C_2H_4, 4-OH, 5-C_2H_5, 6-CH_3			B	R (toluene), C	—
40	1 : 4-CH_3, 2-S·CH_3, 6-O			B	I	—
45	1 : 3-CH_3, 2 : 4-di-O			F	C, m. p. 123—124°	—
78	2-NH_2, 3-C_2H_5, 4-CH_3, 6-O			B	C	—
79	1-C_2H_5, 2-NH_2, 4-CH_3, 6-O			B	—	—

[a] Source of materials.—A: Prof. A. Albert, Australian National University. B: Imperial Chemical Industries Limited, Dyestuffs Division. C: Prof. A. R. Todd, Cambridge University. D: Dr. J. F. W. McOmie, Bristol University. E: Dr. D. J. Brown, Australian National University. F: Genatosan Ltd. G: Dr. N. Whittaker, Wellcome Foundation.

[b] Purity.—C: Chromatographically pure. I: Chromatogram not entirely satisfactory. R: Recrystallised. S: Spectrum unchanged after recrystallisation. The melting points are given in some cases.

RESULTS AND DISCUSSION

The spectra are shown in Figs. 1—16.

(1) *Pyrimidine and Simple Non-tautomeric Derivatives.*—It is impossible at present to attempt a complete assignment of the vibration frequencies of pyrimidine, especially in the absence of Raman data. We can, however, draw some analogies with the related molecules, benzene, pyridine, and deuterobenzene.

The spectrum which we have obtained for pyrimidine agrees generally with that indicated by Brownlie (*loc. cit.*), except that we have found a group of bands near 3050 cm.$^{-1}$ not detailed by him, and also a band at 1610 cm.$^{-1}$ rather than 1650 cm.$^{-1}$, and another at 810 cm.$^{-1}$ rather than 825 cm.$^{-1}$. Our spectrum appears to agree with that recorded earlier by Barnes, Gore, Liddel, and Williams ("Infra-red Spectroscopy," p. 97, Reinhold,

New York, 1944). We found a broad, weak band near 3400 cm.$^{-1}$ as distinct from the strong absorption reported by Brownlie, and it appeared to be due to water absorbed by this hygroscopic substance.

Table 2 gives the values of some ring vibrations together with values for the corresponding modes in the related molecules. Values for benzene, deuterobenzene, and

TABLE 2.

Benzene	Monodeuterobenzene	Pyridine	Pyrimidine
992	981	990	991
1010	1009	1028	1024
1485	1418, 1473	1440, 1485	1400, 1461
1595	1576, 1594	1570, 1580	1569, 1610
1693	—	1723?	1670?

(Frequencies in cm.$^{-1}$)

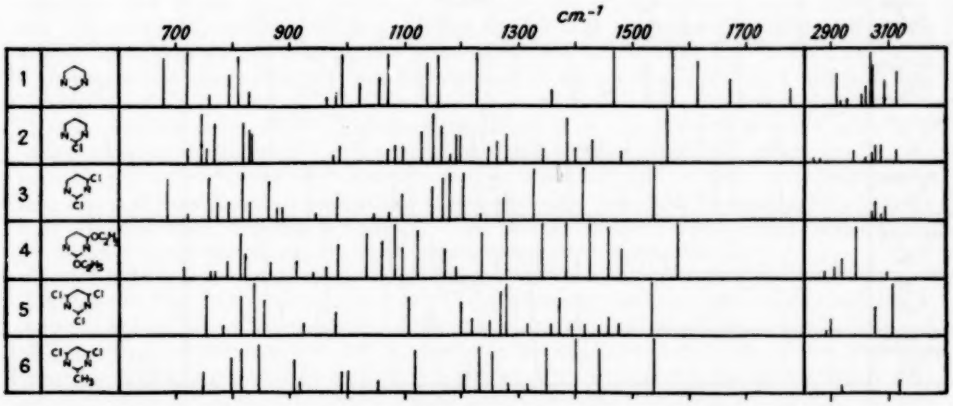
pyridine are taken from the work of Kline and Turkevitch (*J. Chem. Physics*, 1944, **12**, 300). Pyrimidine will possess C_{2v} symmetry, and certain of the twofold degenerate modes in benzene will be split in the same way as in pyridine.

The assignment of frequencies due to bending and deformation of C–H bonds is less obvious, but the following additional similarities are found :

Pyridine	710, 747	1037	1061	1139	1210
Pyrimidine	680, 721	1052	1075	1140, 1165	1220

The spectra of 2-chloro-, 2 : 4-dichloro-, 2 : 4 : 6-trichloro-, 4 : 6-dichloro-2-methyl- and 2 : 4-diethoxy-pyrimidine are compared in Fig. 17.* The most significant feature

FIG. 17.



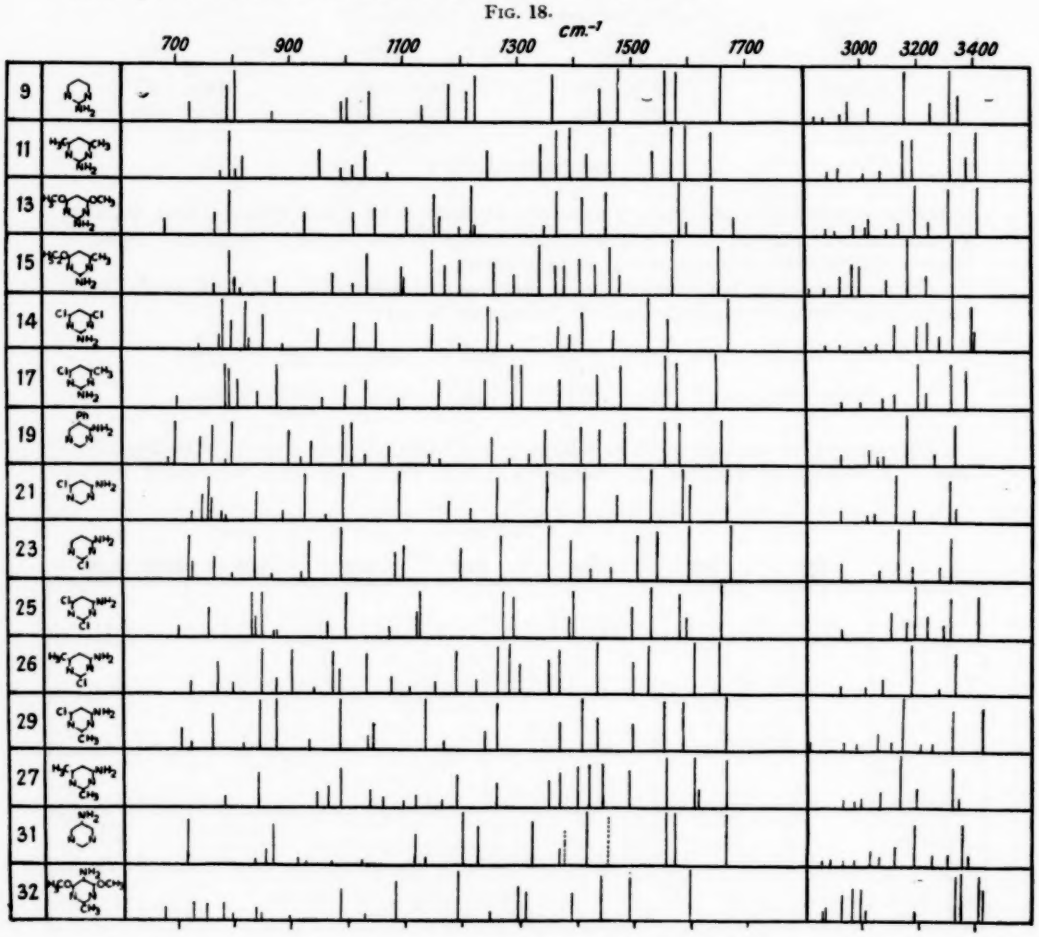
is a strong band in the region 1520—1580 cm.$^{-1}$, which appears to correspond to that at 1569 cm.$^{-1}$ in pyrimidine itself, and has been assigned to a ring vibration. All these compounds have bands near 810 cm.$^{-1}$ and 990 cm.$^{-1}$. The latter is a symmetrical ring vibration, and although the former might have been assigned to a deformation of C–H bonds, its persistence throughout this series rather implies that it may be a ring vibration.

(2) *Monoamino-derivatives.*—The spectra of a number of monoamino-substituted pyrimidines are shown as line drawings in Fig. 18.* All these compounds have marked

* For simplicity the double bonds are not shown in the pyrimidine rings of the formulæ given in Figs. 17—20.

absorption bands at 1500—1700 cm.$^{-1}$ and 3100—3500 cm.$^{-1}$. In the former range of frequency, one or more ring vibrations may occur, corresponding to that near 1550 cm.$^{-1}$ in the simple derivatives discussed above, and deformation vibrations of N–H bonds are also to be expected. The second range of frequency may include certain stretching vibration frequencies of C–H bonds near 3100 cm.$^{-1}$, and the stretching vibration bands of N–H bonds at rather higher frequencies. Combination and overtone bands can occur in both regions, but their relative intensity would probably be low.

FIG. 18.



The spectra as a whole are both complex and individualistic but there are no systematic differences such as to suggest that the 2-amino- and 4-amino-pyrimidines adopt different tautomeric forms. The most marked bands in the higher-frequency range are a pair occurring uniformly near 3150 and 3350 cm.$^{-1}$. These values are rather lower than would be expected for a normal amino-group such as occurs in amines, but they are closely parallel to those near 3180 and 3350 cm.$^{-1}$ found in unsubstituted amides (Richards and Thompson, *J.*, 1947, 1248). If the substituted 2-amino- and 4-amino-pyrimidines exist in the amino-form, and suitable allowance is made for the participating polar structures which are plausible, as well as for the hydrogen bonding, a parallelism with the amides might be expected. The alternative interpretation would be to assign the band at 3150 cm.$^{-1}$ to a C–H vibration, and to suppose that tautomerism in the amino-group has occurred leaving

a side-chain imino-group. This value for the C–H vibration frequency, even in the type of ring concerned, is very high, and this interpretation seems less likely. Moreover, a strong band at 3150 cm.$^{-1}$ only occurs with amino-derivatives. We might alternatively assign it to a ring N–H group, which is carrying a surplus positive charge, somewhat similar to that in amino-acids (Thompson, Nicholson, and Short, *Discuss. Faraday Soc.*, 1950, **9**, 222). This is not in itself very plausible, but in any case the band at 3150 cm.$^{-1}$ is not found with simple hydroxy-pyrimidines in which ketonisation would produce a similar kind of ring NH group.

The most striking feature of the region 1500—1700 cm.$^{-1}$ with monoamino-pyrimidines is a strong band near 1650 cm.$^{-1}$. This lies at higher frequencies than any ring vibration found for the non-tautomeric systems, such as the chloro-derivatives. It is, moreover, removed by deuteration, whereas the other bands from 1500—1600 cm.$^{-1}$ are not much influenced by this treatment. This is further evidence for assigning the band near 1650 cm.$^{-1}$ to a deformation of the amino-group. The similarity with unsubstituted amides is also close in this respect. One or more of the bands between 1500 and 1600 cm.$^{-1}$ will be the analogues of the ring vibration of simple pyrimidines near 1550 cm.$^{-1}$.

By contrast with these simple monoamino-pyrimidines, the 5-amino-4 : 6-dimethoxy-2-methyl derivative shows a markedly different spectrum in the two regions near 6μ and 3μ. This compound can only exist in the amino-form, and its structure is such as to preclude resonance with polar forms. Near 3μ there are strong bands between 3300 and 3430 cm.$^{-1}$ (not at 3150 cm.$^{-1}$), *i.e.* at higher frequencies than with the amino-derivatives just discussed. Also there is no band at 1650 cm.$^{-1}$ corresponding to that suggested already for the deformation of the N–H bond. In this connexion 5-aminopyrimidine is of particular interest since it is almost certainly in the amino-form, amino-imino tautomerism being impossible. The spectrum of this compound in the regions of 3μ and 6μ resembles those of the 2-amino- and the 4-amino-pyrimidines discussed above, and supports the assignment of an amino-structure to these compounds.

When 5-amino-4 : 6-dimethoxy-2-methylpyrimidine is deuterated, the bands near 3μ disappear and others appear near 4μ. Other spectral changes occur below 1300 cm.$^{-1}$, but not noticeably between 1300 and 1700 cm.$^{-1}$. The corresponding nitro-derivative, 4 : 6-dimethoxy-2-methyl 5-nitropyrimidine shows a band at 1571 cm.$^{-1}$ which probably corresponds to that at 1595 cm.$^{-1}$ in the amino-derivatives, and is probably due to a ring vibration.

One peculiar result with the aminopyrimidines is the occurrence in some cases of a strong band near 3400 cm.$^{-1}$, in addition to those between 3150 and 3350 cm.$^{-1}$. This band cannot be correlated with position or type of substituent, or with any other special features in the spectrum of compounds which show it. It might be possible to attribute it to absorbed water, but this is unlikely. The band is almost certainly due to a stretching vibration of N–H bonds, but its exact significance remains obscure.

(3) *Mono-hydroxy-derivatives.*—The spectra of five monohydroxy-pyrimidines are compared in Fig. 19.* Here again there is the possibility of both keto–enol tautomerism and the occurrence of polar forms which may contribute to the final hybrid. Thus, if 2-hydroxypyrimidine exists in the lactim form, we might expect bands of the hydroxyl group, and of the pyrimidine nucleus; if the lactam form occurs, the bands found may simulate those of a monosubstituted amide, with other ring vibrations which may be more or less localised in the C=C and C=N bonds. In either case, the presence of contributing polar forms may affect the situation.

2-Hydroxypyrimidine has a pair of strong bands at 1620—1650 cm.$^{-1}$. In terms of the lactim formula, this absorption would be difficult to explain except as being due to vibrations of the ring, but the values are noticeably higher than those found for ring vibrations in the other simple chloro- or amino-pyrimidines. The alternative would be to explain one band as being due to the deformational vibration of a hydroxyl group, but although the latter seems to vary in frequency in different kinds of molecule, it is improbable that even in the bonded state it could rise to such a high value. A more reasonable interpretation would be to assign one band in this region to the absorption by

* See footnote, p. 181.

a carbonyl group of the lactam form. The apparent doublet at 1620—1650 cm.$^{-1}$ might arise in the lactam form if one ring vibration localised in the C=N bond were raised in value because of changes in bond character resulting from the ketonisation. 2-Hydroxypyrimidine shows no band at frequencies greater than 3200 cm.$^{-1}$, but this does not seem to be conclusive evidence against a lactam form. Monosubstituted amides have a band near 3270 cm.$^{-1}$, but specific conditions of hydrogen bonding could certainly lower this vibration frequency.

FIG. 19.

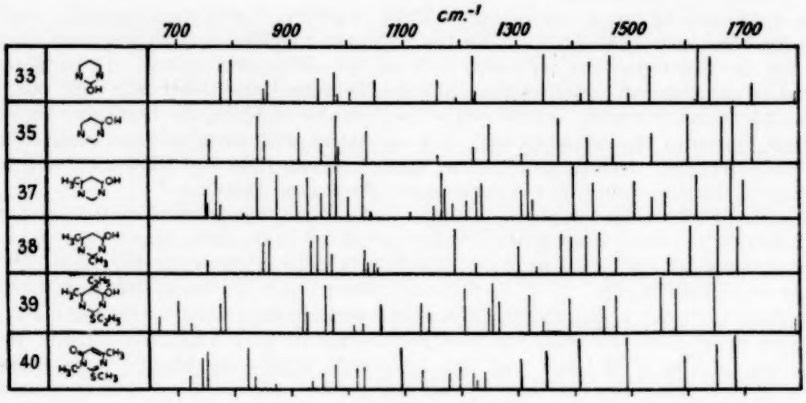
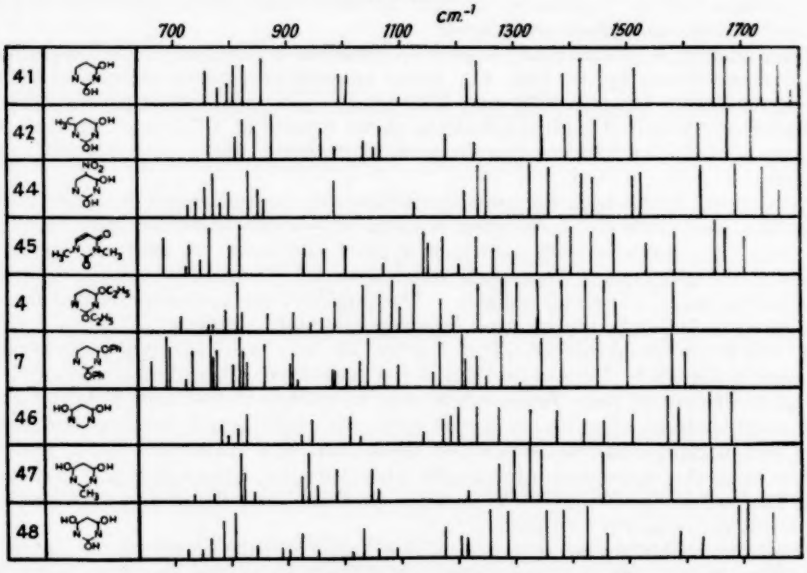


FIG. 20.



Similar arguments apply to 4-hydroxypyrimidine. There are strong bands between 1600 and 1720 cm.$^{-1}$ and their interpretation as ring vibrations of a lactim structure is improbable. It is more reasonable to assume a ketonic formula with ring vibrations raised in value as already indicated. No definite evidence is obtained from the spectrum near 3μ.

Derivatives of 4-hydroxypyrimidine in general behave similarly to the above. It is particularly noteworthy that 1 : 4-dimethyl-2-methylthiopyrimid-6-one is also in line, having strong bands near 1596, 1651, and 1685 cm.$^{-1}$. This compound can only exist in

the ketonic form, and its spectrum in this region is closely parallel to that of 4-hydroxypyrimidine.

(4) *Derivatives with Two or More Tautomeric Groups.*—(a) *Hydroxypyrimidines.* Here the structural problems are even more complex. For example uracil (2 : 4-dihydroxypyrimidine) has six theoretically possible forms, namely the diketonic, dienolic, and four keto–enol forms. The spectra of some hydroxy-derivatives are shown in Fig. 20.*

Uracil has several bands in the range 1640—1770 cm.$^{-1}$ but none between 1510 and 1640 cm.$^{-1}$. By contrast, 2 : 4-diethoxy- and 2 : 4-diphenoxy-pyrimidine have no bands between 1600 and 1800 cm.$^{-1}$. 1 : 3-Dimethyluracil, which must have the lactam structure, has bands between 1640 and 1720 cm.$^{-1}$, which will be due to the carbonyl groups and a possible ring vibration frequency. Partial deuteration of uracil hardly affected the bands at 1653, 1673, 1716, and 1737 cm.$^{-1}$ although that at 1768 cm.$^{-1}$ seemed to disappear. This evidence suggests that uracil exists in a form which is at least partly ketonic, and probably diketonic. On the other hand, the change in the spectrum of uracil between 700 and 1500 cm.$^{-1}$ after deuteration is remarkable, and much more pronounced than with most other compounds. Uracil has a strong band near 1230 cm.$^{-1}$ which is removed by deuteration, and might be attributed to a hydroxyl group.

6-Methyl- and 5-nitro-uracil also each have two strong bands in the region 1670—1740 cm.$^{-1}$ and a third near 1630 cm.$^{-1}$. These compounds do not show a band near 1580 cm.$^{-1}$ found with some derivatives and thought to be a ring frequency.

None of the derivatives of uracil has strong bands above 3200 cm.$^{-1}$ and the feebler bands below this frequency cannot be definitely assigned.

4 : 6-Dihydroxypyrimidine shows a marked spectral difference near 6μ from the 2 : 4-dihydroxy-derivative. It has three strong bands near 1570, 1641, and 1681 cm.$^{-1}$ with a weaker band at 1588 cm.$^{-1}$. There is a close parallelism with 4 : 6-dihydroxy-2-methylpyrimidine; by contrast with uracil there is no band above 1700 cm.$^{-1}$. 2-Methylthio-1 : 6-dimethylpyrimid-6-one, which contains a single carbonyl group, is again closely similar to this respect to 4 : 6-dihydroxypyrimidine, and all this evidence may be consistent with its being singly ketonic, *i.e.*, with one carbonyl and one hydroxyl group. Indirectly, this may support the argument that uracil is diketonic. Incidentally, 4 : 6-dihydroxypyrimidine has a band near 3300 cm.$^{-1}$ which could be due to a bonded-hydroxyl group, although no corresponding band is found with 4 : 6-dihydroxy-2-methylpyrimidine.

One feature of the spectra between 700 and 1500 cm.$^{-1}$ which may serve to differentiate the 2 : 4- and the 4 : 6-dihydroxy-derivative is the occurrence of a fairly marked band near 760 cm.$^{-1}$ with the former class, but not with the latter.

2 : 4 : 6-Trihydroxypyrimidine (barbituric acid) has at least three intense bands in the region 1690—1760 cm.$^{-1}$; these bands are again attributed to carbonyl groups. While the spectrum would be consistent with a structure involving two carbonyl groups and one hydroxyl group, it is impossible to infer this directly. In the region of 3μ the spectrum is somewhat similar to that of 4 : 6-dihydroxypyrimidine, having a pair of strong bands at 3087 and 3204 cm.$^{-1}$. The latter of these could be due to a bonded-hydroxyl group, although again its assignment to an imino-group cannot be excluded.

(b) *Amino-derivatives.* Some di- and tri-aminopyrimidines have been examined. All show a complex group of strong bands at 1400—1650 cm.$^{-1}$, assignment of which is difficult. There are three strong bands at 3125, 3300, and 3450 cm.$^{-1}$. The monoamino-derivatives usually show the first two of these bands, but not that at 3450 cm.$^{-1}$. All three are probably due to stretching vibrations of N–H bonds, and in this connexion Clews and Cochran's *X*-ray measurements (*Acta Cryst.*, 1948, **1**, 4; 1949, **2**, 46) on 4 : 6-diamino-5-bromopyrimidine are interesting, since it was found that the two amino-groups are not equivalent, but bonded differently to adjacent molecules in the crystal. This might account for the spectral differences found between the monoamino- and diamino-derivatives.

(c) *Derivatives with hydroxyl and amino-groups.* Three types of compound containing one amino- and one hydroxy-group have been studied, namely derivatives of 2-amino-4-hydroxy-, 2-hydroxy-4-amino-, and 4-hydroxy-6-amino-pyrimidine.

2-Amino-4-hydroxy-6-methylpyrimidine has strong bands near 1500 cm.$^{-1}$ and

* See footnote p. 181.

1659 cm.$^{-1}$. Partial deuteration leaves the former unchanged and the latter appears to be replaced by a pair at 1605 and 1639 cm.$^{-1}$. The band at 1659 cm.$^{-1}$ may in reality be a double band, one component being shifted to 1605 cm.$^{-1}$. This compound has strong bands at 3068 and 3332 cm.$^{-1}$, corresponding to those at 3160 and 3320 cm.$^{-1}$ with most 2-amino-derivatives, including 2-amino-4-ethoxy-6-methylpyrimidine, and these bands must be due to N–H stretching vibrations. On balance the spectra perhaps favour a form in which the hydroxyl group is ketonised and the amino-group remains unchanged except for hydrogen bonding.

2-Amino-4-hydroxy-6-methyl- and 2-amino-4-ethoxy-6-methyl-pyrimidine have strong bands at 1500—1700 cm.$^{-1}$. In the former case, the band at 1649 cm.$^{-1}$ is probably due to a carbonyl group and is not much affected by deuteration, but the latter compound also has a band near 1650 cm.$^{-1}$ which is removed by deuteration. It is therefore clear that we have a number of overlapping or close-lying bands due to carbonyl and N–H bond vibrations, and possibly also to ring vibrations. The bands near 3μ previously attributed to the amino-group are absent from the spectrum of the dimethylamino-derivatives.

Three compounds were measured in which the amino- and hydroxyl groups occupied the 4- and the 6-position with a non-tautomeric substituent in the 2-position. These were 6-amino-4-hydroxy-, 6-amino-4-hydroxy-2-methylthio-, and 6-amino-4-hydroxy-2-methyl-pyrimidine. In the region 1500—1700 cm.$^{-1}$ a close similarity between the spectra might have been expected, but in fact the strong bands occur at noticeably different positions. With 6-amino-4-hydroxypyrimidine the band at 1664 cm.$^{-1}$ is removed by deuteration, indicating that it is due to N–H or O–H, and by analogy with previous results the former alternative would be expected. In that case, the band at 1610 cm.$^{-1}$ would have to be assigned to the carbonyl group, and this seems an extremely low value although hydrogen bonding might lead to a fall below normal. With 6-amino-4-hydroxy-2-methylpyrimidine a band at 1676 cm.$^{-1}$ is removed by deuteration, and another near 1625 cm.$^{-1}$ persists. A corresponding interpretation might be given, but there is always the possibility that the bands just above 1600 cm.$^{-1}$ may be due to ring vibrations. In the region of 3μ, both the 6-amino-4-hydroxy- and 6-amino-4-hydroxy-2-methyl derivatives have strong bands near 3140 cm.$^{-1}$ and 3340 cm.$^{-1}$, and the 6-amino-4-hydroxy-2-methylthio-derivative has a more complex absorption with bands at higher frequencies. These differences must again originate in differences of polar character of groups, and from special interactions. It therefore seems impossible to decide whether the 6-amino-4-hydroxypyrimidines have a keto-amino-structure or remain in the enolic form.

The spectrum of 4-amino-2-hydroxypyrimidine agrees essentially with that reported by Blout and Fields (*J. Amer. Chem. Soc.*, 1950, **72**, 479), but we have obtained better resolution. These workers assigned the bands at 3425 cm.$^{-1}$ and 3205 cm.$^{-1}$ to O–H and N–H vibrations, but as already stated they are probably more satisfactorily explained as being due to the amino-group as in a solid unsubstituted amide group. There are strong bands at 1507, 1541, and 1659 cm.$^{-1}$, similar to those of 2-hydroxypyrimidine, and the changes produced by deuteration are similar. The evidence is consistent with the occurrence of a keto-amino-form.

The spectrum of 6-amino-2 : 4-dihydroxypyrimidine resembles that of the 2 : 4-dihydroxy-6-methyl derivative between 1500 and 1700 cm.$^{-1}$, and both may exist in the diketonic form. 2-Amino-4 : 6-dihydroxypyrimidine has a strong band near 1680 cm.$^{-1}$ which is absent with the corresponding dimethoxy-derivative and the spectrum is more like that of 4 : 6-dihydroxypyrimidine, possibly a ketonic–enolic structure.

2 : 4-Diamino-6-hydroxy- and 4 : 6-diamino-2-hydroxy-pyrimidine differ from each other in the region of 6μ, although they resemble respectively the 6-hydroxy(4-hydroxy)- and 2-hydroxy-derivatives. There is a difference near 3μ between 4 : 6-diamino-2-hydroxy- and 6-hydroxy-2 : 4-diamino-pyrimidine in that the former has a strong band near 3035 cm.$^{-1}$ in addition to the three bands at higher frequency given by both. A parallel difference arises between 2-hydroxy- and 4-hydroxy-pyrimidine. Although therefore the bulk of the evidence may favour a ketonic form for both 2-hydroxy- and 4-hydroxy-derivatives, there may be a difference between hydroxyl groups in the 2- and the 6-position in some cases.

(d) *Mercaptopyrimidines.* Neither 2-methylthio- nor 2 : 4-dimethylthio-pyrimidine has

bands between 1625 and 1800 cm.$^{-1}$. Comparison of the former with 2-hydroxypyrimidine shows that the latter has a band at 1647 cm.$^{-1}$ in addition to that at 1625 cm.$^{-1}$. This again suggests that 2-hydroxypyrimidine is in the ketonic form. An intense band occurs with 4-hydroxy-2-methylthiopyrimidine at 1695 cm.$^{-1}$, corresponding to that found with 4-hydroxypyrimidine itself. However, no band is found near this frequency with either 4-hydroxy-2-methylthio-6-methyl- or 6-amino-4-hydroxy-2-methylthio-pyrimidine, in spite of a general agreement between the spectra. This would imply that either in these latter compounds the structure is enolic, or the carbonyl group band is weakened or displaced.

(5) *Spectra in the Region* 15—25μ.—Some of the compounds were examined in this range, and the results are shown in the diagrams. Although there are no obvious structural correlations, it seems that this spectral region might well prove valuable for analytical work and for identification.

Conclusions

The complexity and ambiguities of the interpretations given above make it desirable to summarise the main conclusions, as follows :

(i) In the hydroxy- and amino-pyrimidines, either alone or with the addition of other substituents around the pyrimidine ring, the main spectral regions of interest as regards the tautomerism which may occur are at 6μ and 3μ. Unfortunately in both these regions certain ambiguities limit the correlation of bands with structure. Near 6μ, bands are to be expected due to bending modes of N–H bonds, to carbonyl groups, and to ring vibrations. The variation in frequency of these key bands with differences of polar character of bonds and special interactions are such as to preclude many definite assignments. Further, the intensity of ring vibrations of the kind concerned is known, with simple derivatives of benzene for example, to vary from one compound to another in a somewhat unpredictable way. In the region of 3μ, too, ambiguities arise in assigning the stretching vibration frequencies of different kinds of N–H and O–H bond. For all these reasons, it would be unwise to be too dogmatic in making frequency assignments.

(ii) The spectra of different derivatives of pyrimidine are highly characteristic, and since most of the bands are sharp, they might be useful for analysis and identification even though precise structural correlations are obscure.

(iii) It is suggested that the balance of the spectral evidence favours a ketonic structure for the simple 2-hydroxy- and 4-hydroxy-pyrimidines, and probably a diketonic form for the 2 : 4-dihydroxy-derivatives. In 4 : 6-dihydroxypyrimidine one ketonic and one enolic group may be present.

(iv) Amino-substituents are thought in general to exist in the non-tautomerised form, and to have the character of an amido-group.

(v) The electronic effects of different substituent groups may influence the tautomerism and also the quantitative degree of interaction through hydrogen bonding.

(vi) Our conclusions do not agree in some respects with those of Brownlie (*loc. cit.*). He did not measure the simple monohydroxy- and monoamino-derivatives, and other compounds in which tautomerism is prevented by appropriate substitution. These latter measurements provide important evidence. Brownlie appears to have accepted an enolic form and we do not think that his assignment of bands near 3150 cm.$^{-1}$ to the hydroxyl group is satisfactory. Also, in arguing for the imino-structure of certain derivatives, he based some conclusions on the occurrence of only one N–H stretching vibration near 3μ, whereas our results with the same compound indicated a more complicated spectrum in this region. We feel that a clear-cut assignment of frequencies in the way proposed by Brownlie is impossible, and that variations with different derivatives may be marked. If the details of structure of a few simple hydroxylic and amino-derivatives could be established by *X*-ray measurements, so that correlations could be made with the spectrum in these cases, it might assist in a more general interpretation.

We are greatly indebted to those listed above for giving us specimens of pure compounds, and particularly to Professor A. R. Todd, F.R.S. One of us (L. N. S.) also thanks the Australian National University for a scholarship.

The Physical Chemistry Laboratory, Oxford. [*Received, July 24th*, 1951.]

39. *Photochemical Studies of Some Organic Nitrogen Derivatives.*

By G. H. Booth and R. G. W. Norrish.

An investigation has been made of the products of decomposition of solutions of some organic nitrogen derivatives in the full light, and in filtered light from a hot mercury arc. Particular attention has been given to gaseous decomposition products and to the development of unsaturation in paraffinoid solvents accompanying the decomposition.

It has been demonstrated that aliphatic primary and secondary amines, and ammonia itself, give products arising mainly from a primary photochemical act which involves the rupture of a nitrogen–hydrogen bond to give free radicals.

Experiments on aliphatic amides show that, in general, two main types of primary photochemical process occur. Both of these are of a molecular rather than of a free-radical nature. A close analogy is drawn between these reactions and the behaviour of the aldehydes and ketones under similar conditions.

Some extension of the work to the examination of the photolysis of proteins has been attempted.

It was observed by L. Farkas (*Z. physikal. Chem.*, 1933, *B*, **23**, 89) that, when a solution of ammonia in hexane is irradiated by the light from a mercury arc, the only gaseous product obtained is hydrogen. He ascribed this to a photodecomposition of the hexane, sensitised by the ammonia. The wave-length of the light required to bring about this reaction corresponds with the pre-dissociation region of ammonia. As the effect of light of this wave-length almost certainly involves the breaking of a N–H bond in the ammonia molecule, it was considered by Bamford and Norrish (*J.*, 1938, 1531), in the light of their investigation of the photolysis of aldehydes and ketones in the gas phase and in paraffinoid solution, that the ammonia reaction is not a true photosensitisation, but rather the result of reactions based on a primary photochemical act represented by :

$$NH_3 + h\nu \longrightarrow NH_2 + H$$

Subsequent work by Bamford (*J.*, 1939, **17**) on the photodecomposition of aliphatic amines in the gas phase has added weight to this view. Here the primary act has been found to be :

$$R{\cdot}NH_2 + h\nu \longrightarrow R{\cdot}NH + H$$

This has been supported more recently by Wetmore and Taylor (*J. Chem. Physics*, 1944, **12**, 61) and Johnson and Taylor (*ibid.*, 1951, **19**, 613).

In the first part of the present work, the aim has been to substantiate this idea by an examination of the photochemical reactions undergone by both ammonia and the amines in hydrocarbon solution. Later, the work was extended to the investigation of similar reactions of amides, of which very little has hitherto been recorded. These compounds have been studied in hexane and in dioxan solution.

The power of the method lies in the great simplification of the dark reactions undergone by the entities produced by the primary photochemical act. In particular, a free radical produced in a hydrocarbon solution is most readily hydrogenated at the expense of the solvent, thereby giving a simple hydrocarbon radical, the ultimate fate of which may be easier to envisage.

The general conclusions drawn are that we have a series of reactions remarkably similar to those undergone by aldehydes and ketones. Evidence is cited for two main types of primary reaction.

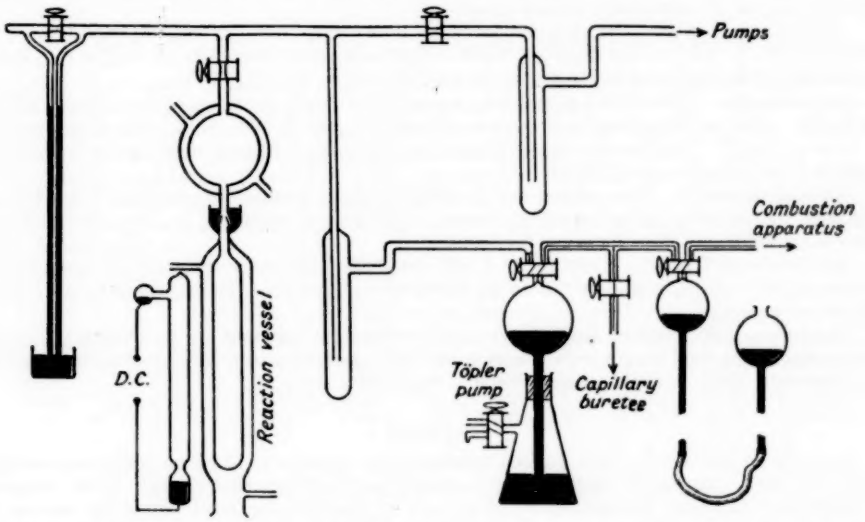
Some attempt has also been made to extend the method and general ideas to the investigation of the behaviour of proteins under ultra-violet radiation. Owing to the complexity of the starting materials, however, only very general inferences may be drawn from the results obtained.

Experimental

The irradiation of the reaction systems was carried out in a double-walled cylindrical quartz vessel. The apparatus is shown diagrammatically in the figure. The space between the two walls of the reaction vessel was used for the circulation round the reaction mixture of water at controlled temperatures or of liquid colour filters. The inner compartment of the reaction vessel was connected to a conventional high-vacuum system and to a Töpler pump, by means of which gaseous reaction products could be removed for analysis.

Before irradiation, the reaction vessel and its contents were thoroughly " degassed," until nothing could be extracted by the Töpler pump, while the reaction vessel was immersed in liquid nitrogen.

The reaction mixture was illuminated by a hot mercury arc running at about 400 w. Only that part of the reaction vessel containing liquid phase was exposed, vapour above and (in some cases) solid below the liquid being carefully screened from the light.



After some hours' irradiation, the lamp was removed, the thermostat jacket drained, and a liquid-nitrogen bath placed round the reaction vessel. Gaseous reaction products were then removed for analysis through a liquid-nitrogen trap by means of the Töpler pump. This process was repeated after the system had been allowed to " degas " again. Whenever possible, further gaseous products were extracted, the reaction vessel being held at −80° in an acetone–carbon dioxide freezing mixture. These gases were examined separately from those removed at −196°.

Examination of Products.—(a) *Gases.* Unsaturated hydrocarbon gases (except ethylene) were determined by absorption in concentrated sulphuric acid. The method used was a capillary burette method based on that of Swearingen, Gerbes, and Ellis (*Ind. Eng. Chem., Anal.*, 1933, **5**, 369). Ethylene was determined similarly by absorption in fuming sulphuric acid.

Carbon monoxide, hydrogen, and methane were determined by combusion with oxygen on a heated platinum spiral in an apparatus similar to that used by Axford and Norrish (*Proc. Roy. Soc., A*, 1948, **192**, 518). From pressure changes in a known volume, as recorded by a sensitive Foord-type collapsed spoon gauge (*J. Sci. Inst.*, 1934, **11**, 126), the total change in pressure on combustion, the oxygen consumed, and the carbon dioxide produced could be computed. Hence, a three-component gaseous mixture could be fully analysed.

(b) *Liquid products.* Non-volatile products of reaction, and unchanged non-volatile reactants, were removed by distilling and collecting the volatile portion of the material, including the solvent, left in the reaction vessel. This distillation was done *in vacuo* into liquid nitrogen. For reactions in hydrocarbon solution, the distillate was washed with dilute acid and several lots of water. The washings were retained and examined separately. The

washed hydrocarbon was examined for unsaturation by keeping it for some time with a known amount of standard bromine water and back-titrating the excess (*via* potassium iodide) with standard thiosulphate. The method was tested upon known solutions and found to be effective.

The washings from hydrocarbon solutions and the whole of residual dioxan solutions were examined qualitatively only for the presence or absence of various compounds, the formation of which might be inferred from a consideration of the gaseous products obtained.

Preparation and Purification of Reagents.—(a) *Hexane.* Commerical *n*-hexane (b. p. 67—69°) was used as starting material. This was shaken repeatedly with small portions of warm concentrated sulphuric acid (*d* 1·83) until the acid was no longer discoloured after contact with the hydrocarbon. This treatment serves to remove unsaturated constituents. To remove aromatic constituents, the hydrocarbon was allowed to run through a 1·5-ft. column of silica gel. The liquid, after being washed with dilute sodium hydroxide and water, was then dried ($CaCl_2$; Na) and redistilled, a large middle cut being collected. A small amount of residual unsaturation, not removed by repetition of the above process, was determined quantitatively and allowed for in subsequent measurements.

(b) *Dioxan.* Dioxan was refluxed for some hours with metallic sodium, then filtered and redistilled (b. p. 101°). The resultant liquid, which was stored over reduced copper to minimise peroxidation, was transparent to ultra-violet light down to 2350 Å.

(c) *Ammonia.* Gaseous ammonia was generated by gentle warming of the aqueous solution (*d* 0·880). The gas was passed successively through a vessel held at 0°, a quick-lime tower, and a trap at −10°. It was then either passed directly into a hexane saturator or collected in solid form in a liquid-nitrogen trap.

(d) n-*Hexylamine.* The amine was synthesised from *n*-hexyl bromide by the Hofmann method, the primary amine being separated. The final product, b. p. 130—132°/750 mm., gave a negligible reaction for secondary amine.

(e) *Ethylmethylamine.* A sample, b. p. 35°, was redistilled twice *in vacuo* into acetone–solid carbon dioxide traps, a middle cut being taken at each stage. The liquid gave no reaction for primary amines.

(f) *Amides.* A range of amides were recrystallised to constant m. p. from acetone–light petroleum. The final m. p.s were: acetamide, 81°; propionamide, 79°; *n*-butyramide, 115°; *n*-valeramide, 106°; *n*-hexanoamide, 100·5°; stearamide 108°.

Results

Samples of the hexane and dioxan solvents were irradiated alone as blank experiments. After 10 hours' photolysis at 40°, a very small amount of gas (not enough to be measured) could be removed from the hexane. The amount of unsaturation developed in the hexane was likewise negligible, being equivalent to less than 1 drop of N/100-bromine. No gas was obtained from the dioxan.

Ammonia.—The photolysis of ammonia was studied in hexane solution only. The system was made up in two ways as follows:

(a) *Low concentration of ammonia.* The ammonia, prepared as above, was allowed to bubble in a slow stream through hexane held at 0° for about an hour. 50 Ml. of the solution were measured into the reaction vessel and degassed as described, the liquid-nitrogen bath holding back the ammonia in the hydrocarbon. The final pressure of ammonia over the solution was 20 mm. Hg. The solution was irradiated at 20°, the results being given in Table 1. The unsaturation developed in the solvent is expressed as ml. of gaseous olefin, on the assumption that the olefin behaves as an ideal gas.

TABLE 1.

Temp. = 20°.

Time of irradiation (hours)	H_2 obtained (ml. at N.T.P.)	Olefin in hydrocarbon (ml. at N.T.P.)
6	0·98	0·95
7	1·03	0·92
6	0·62	0·62
10	0·66	0·68

(b) *High concentration of ammonia.* 50 Ml. of hexane were thoroughly degassed in the reaction vessel, and excess of solid ammonia sublimed in. The whole system was allowed to warm to room temperature, the excess of ammonia escaping from the foot of a mercury

manometer. The whole system was then refrozen in liquid nitrogen to give intimate mixing of the two constituents. After the mixture had been allowed to warm a second time, the final total pressure in the reaction system was adjusted to something under atmospheric by drawing off a little vapour with the pumps. The procedure was then as before. Table 2 presents the results.

TABLE 2.

Temp. = 20°.

Total pressure over solution (mm. Hg)	Time of irradiation (hours)	H_2 (ml.)	Unsaturation (ml.)
480	6	0·98	0·99
740	6	0·42	0·49
740	20	0·80	0·78
740	18	0·68	0·67

(c) A run, taken in a similar manner to those in (b) above, but in which the vapour phase only was irradiated, the liquid being shielded from the light, gave the following results :

Temp. = 20°. Time of photolysis = 12 hours.
H_2 obtained = 0·12 ml. Unsaturation in liquid at end = 0·14 ml.

Amines.—(a) n-*Hexylamine in hexane.* Results for this system were obtained under three different sets of conditions, namely : (i) Amine concentration varying; temperature and time of photolysis constant. (ii) Temperature varying; concentration and time constant. (iii) Time of photolysis varying; concentration and temperature constant.

The results are given in Tables 3, 4, and 5 respectively. During these runs, great care was taken to place the lamp as nearly as possible in the same position with respect to the reaction vessel. Even so, owing to variation in the mains supply to the lamp, constant intensity cannot be claimed.

TABLE 3.

Temp. = 20°. Time of photolysis = 10 hours.

Concn. of amine (mole/l.)	H_2 (ml.)	Unsatn. (ml.)	Ratio, unsatn./H_2	Concn. of amine (mole/l.)	H_2 (ml.)	Unsatn. (ml.)	Ratio, unsatn./H_2
0·760	7·38	2·79	0·38	0·076	2·39	2·27	0·95
0·302	5·36	2·36	0·44	0·038	1·60	1·61	1·00
0·151	3·53	2·15	0·61	0·015	0·11	0·11	1·00

TABLE 4.

Amine concn. = 0·038 mole/l. Time of photolysis = 10 hours.

Temp.	H_2 (ml.)	Unsatn. (ml.)	Ratio, unsatn./H_2	Temp.	H_2 (ml.)	Unsatn. (ml.)	Ratio, unsatn./H_2
40°	4·62	2·14	0·46	30°	3·92	1·95	0·50
40	3·93	1·88	0·48	20	3·38	2·30	0·68
40	4·60	2·23	0·48	20	3·06	2·09	0·68
30	3·97	2·24	0·56	13	1·60	1·6	1·00
30	5·00	3·19	0·56	2	1·47	1·47	1·00

TABLE 5.

Amine concn. = 0·038 mole/l. Temp. = 30°.

Time of photolysis (hours)	H_2 (ml.)	Unsatn. (ml.)	Ratio, unsatn./H_2	Time of photolysis (hours)	H_2 (ml.)	Unsatn. (ml.)	Ratio, unsatn./H_2
10	5·00	3·19	0·56	3·75	0·67	0·53	0·79
7·5	2·55	1·48	0·58	2·5	0·52	0·49	0·94
5	0·91	0·61	0·67				

The variation of the ratio, liquid unsaturation : hydrogen, with concentration, temperature, and time is indicated in the final columns of these tables. It was observed in these experiments that, when the value of this ratio was less than unity, a yellow coloration appeared in the liquid during the course of the run. On extraction of the amine from the hydrocarbon with dilute acid, at the end of the run and before measurement of unsaturation, a brown scum collected at the interface between the organic and the aqueous layer. When hydrogen and unsaturation balanced, no such phenomenon was observed. This brown material, once having collected at the interface, could not be got back into solution either in hydrocarbon or in water. An examination of this " sludge " disclosed a positive reaction in the Lassaigne test for nitrogen.

The material always consumed bromine from bromine water, but the consumption was never sufficiently reproducible to allow quantitative assessment.

Nessler reagent tests for ammonia in the acid extract from the hydrocarbon were not conclusive, owing to interference by the amine which gives a copious white precipitate. However, some inference may be drawn from the fact that, when 5 ml. of the extract were contaminated with 1 ml. of solution containing $3{\cdot}01 \times 10^{-4}$ mole of NH_3 per l., the red-brown ammonia colour with Nessler reagent became apparent at once. A run on the hexylamine–hexane system at 40° at an amine concentration of 1·52 mole/l. gave in 6 hours 2·53 ml. of hydrogen. After removal of this gas, the residual solution, which now contained a visible amount of the brown by-product was set aside in the dark at 40° for a further 7 hours. An additional 0·12 ml. of hydrogen was then obtained. A Nessler reagent test on the dilute acid extract from the remaining mixture in the reaction vessel gave negative results for ammonia.

(b) *Ethylmethylamine in hexane.* Experiments on this system were conducted as for hexylamine, with an examination of the concentration effect only. The results are presented in the following table.

Temp. = 20°. Time of photolysis = 14 hours.

Concn. of amine (mole/l.)	H_2 (ml.)	CH_4 (ml.)	Unsatn. (ml.)	Ratio $\frac{\text{unsatn.}}{\text{total gas}}$
0·234	8·07	0·64	3·31	0·38
0·117	5·55	0·30	2·50	0·43
0·058	4·74	0·07	2·64	0·55
0·029	2·18	0·07	1·83	0·81
0·015	1·23	—	1·22	0·99

In no case could ethane be withdrawn from the reaction mixture, even at −80°.

A run on this amine in the gas phase at 20° in the presence of hexane vapour gave, in 5 hours, 1·92 ml. of hydrogen and 0·01 ml. of methane.

Brown insoluble material separated in all these runs in the same circumstances as with hexylamine. In the gas-phase reaction, the " sludge " was deposited as a thin film on the walls of the reaction vessel.

Amides.—(a) *With dioxan as solvent.* Owing to the very low solublity of the amides in hydrocarbons, it was decided to use dioxan as a solvent, in order to have a solution giving a large absorption and hence probably an increased yield of products. This substance, transparent to ultra-violet light down to 2350 Å, has the further advantage of being rich in hydrogen for the hydrogenation of free radicals.

(i) n-*Hexanoamide in dioxan.* A series of experiments with solutions of *n*-hexanoamide of varying concentration in dioxan at 40° gave the results presented in the annexed table.

Temp. = 40°.

		CO		H_2	
Concn. of amide (mole/l.)	Time of photolysis (hours)	(ml.)	(%)	(ml.)	(%)
0·337	17	0·810	79	0·210	21
0·241	19	1·060	69	0·470	31
0·172	10·5	0·320	69	0·145	31
0·172	11·5	0·375	66	0·190	34
0·139	12	0·630	66	0·325	34
0·138	13	0·380	53	0·340	47
0·104	14·5	0·755	65	0·415	35
0·046	17	0·530	65	0·285	35
0·037	16	0·750	63	0·445	37
0·017	10	0·505	62	0·310	38
0·003	24	1·735	69	0·760	31

The residual solution, on being tested with Nessler reagent, gave a yellow colour indicative of the presence of amines, in addition to the large white precipitate due to unchanged amide. The Schiff's reagent test for aldehydes and the *o*-phenylenediamine test for α-diketones both gave negative results.

(ii) n-*Butyramide in dioxan.* Some results for this system, obtained in the same way as above, are given in the following table.

Temp. = 40°.

		CO		H_2	
Concn. of amide (mole/l.)	Time of photolysis (hours)	(ml.)	(%)	(ml.)	(%)
0·090	11	0·665	74	0·230	26
0·043	14	0·355	54·5	0·295	45·5
0·007	10	0·360	59	0·250	41

(iii) ***Propionamide in dioxan.*** A concentrated solution of propionamide was irradiated at 40° for 8½ hours. No gas could be pumped away at liquid-nitrogen temperatures, but at −80° 0·505 ml. of gas was obtained, which analysed as ethylene.

(iv) ***Acetamide in dioxan.*** Some results for strong solutions of acetamide at 40° are shown in the following table. A point to be noted here is that no methane at all was obtained.

	CO		H_2	
Time of photolysis (hours)	(ml.)	(%)	(ml.)	(%)
12	0·630	71·5	0·250	23·5
16	0·905	75	0·300	25

(b) ***Experiments in hexane solution.*** A knowledge of the gaseous decomposition products of the amides now having been obtained, examination of hydrocarbon solutions was undertaken. A rough measurement of the solubility of *n*-hexanoamide in hexane gave a value of about 0·34 g./l. at 40°. Solutions saturated at the working temperature were used in the ensuing experiments. Excess of solid amide was kept in the bottom of the reaction vessel, so as to keep up the concentration as reaction proceeded, and was screened from the light.

(i) n-***Hexanoamide in hexane.*** Results at two temperatures are given in the annexed table. In all cases, the unsaturation developed in the hexane was in excess of that required for balance with the hydrogen evolved. A column is therefore included under the heading of " unsaturation minus hydrogen." The gases analysed were all drawn off at −196°.

In all cases, amines were detected in the acid extract from the volatile portion of the final liquid mixture, their presence being indicated by a pale yellow precipitate with Nessler reagent. Evaporation of the extract gave a very small crop of crystals of indefinite m. p., which gave positive reaction in the carbylamine test for primary amines. The Schiff and the *o*-phenylenediamine test both gave negative results.

Temp.	Time (hours)	CO (ml.)	H_2 (ml.)	Unsatn. (ml.)	Unsatn. − H_2 (ml.)
40°	28·5	0·270	0·690	0·79	0·10
40	13	0·150	0·585	0·67	0·085
40	10·5	0·100	0·380	0·39	0·01
40	12	0·420	1·500	1·89	0·39
30	16	0·190	0·720	0·82	0·10
30	16	0·205	1·450	1·66	0·21
30	12	0·130	0·860	0·96	0·10

In some runs, the detailed results of which are not recorded, some unsaturated gas, completely soluble in concentrated sulphuric acid, was drawn off at −80°. The amount was, however, never sufficient to account for all the " extra " unsaturation. In the one case in which an amount enough for a combustion analysis was obtained, the gas burned as $CH_{2\cdot2}$.

(ii) n-***Valeramide in hexane.*** Results of experiments comparable to those above are given below. Gaseous product, withdrawn at −80° and completely soluble in concentrated sulphuric acid, is entered as " gaseous unsaturation." Amines were again found in the acid extract.

	Temp. = 40°.			
Time of photolysis (hours)	CO (ml.)	H_2 (ml.)	Gaseous unsatn. (ml.)	Liquid unsatn. (ml.)
14	0·200	0·425	0·110	0·42
16	0·215	0·565	0·112	0·58

(iii) n-***Butyramide in hexane.*** In this case, the extra gas obtained at −80° was not soluble in concentrated sulphuric acid, but did dissolve in the fuming acid. It is entered in the table, with the other results, as ethylene.

	Temp. = 40°.			
Time of photolysis (hours)	CO (ml.)	H_2 (ml.)	C_2H_4 (ml.)	Liquid unsatn. (ml.)
11·5	0·115	0·425	0·056	0·43
14·5	0·335	0·460	0·151	0·46

(iv) ***Propionamide in hexane.*** A saturated solution was irradiated for 10 hours at 40°. The gas obtained at −196° analysed as 0·065 ml. of carbon monoxide and 0·340 ml. of hydrogen. A further 0·120 ml. was obtained at −80° and analysed as ethylene. After extraction of the hydrocarbon solvent, an unsaturation value of 0·34 ml. of olefin was found.

(v) *Acetamide in hexane.* Again in the case of acetamide, no extra gas was obtained at −80° and no methane was included among that withdrawn at −196°. The results were as follows:

Temp. = 40°.

Time of photolysis (hours)	CO (ml.)	H_2 (ml.)	Liquid unsatn. (ml.)
14·5	0·260	0·860	0·83
16	0·075	0·390	0·42
16	0·170	0·625	0·62

(vi) *Stearamide in hexane.* No gas condensable at −196° but volatile at −80° was obtained. The results were as follows:

Temp. = 40°.

Time of photolysis (hours)	CO (ml.)	H_2 (ml.)	Unsatn. (ml.)	Unsatn. − H_2 (ml.)
14	0·130	0·335	0·43	0·09
17	0·125	0·240	0·30	0·06

(vii) *Stearamide in medicinal paraffin.* A sample of medicinal paraffin was purified by Bamford and Norrish's method (*loc. cit.*), in order that some experiments might be conducted on solutions at 100°. It was found impracticable, however, for all amides except stearamide, owing to volatilisation. The solutions were all stripped of solute before sufficient photolysis had occurred, the amides collecting as crystals in the cooler and screened parts of the apparatus. Also, a slow progressive and irreproducible decomposition of the solvent itself occurred after about 8 hours' irradiation.

A solution of stearamide, however, gave, in 7 hours at 100°, 0·055 ml. of carbon monoxide and 0·230 ml. of hydrogen. Measurement of unsaturation was not possible, as the solvent and unchanged solute could not be separated.

Effect of Colour Filters on the Amide Reaction.—The hexanoamide–hexane system was studied at 40°, both in the full light of the arc and with a range of filters interposed between the lamp and the reaction vessel. These filters were all liquids with the exception of one of Pyrex glass. The results appear in the annexed table. The transmission limits of the filters were obtained by examination in a Beckman D.U. quartz spectrophotometer.

Filter	Short wave-length cut-off	H_2 (ml.)	CO (ml.)	Unsatn. (ml.)
—	<2500	1·500	0·420	1·89
1% Aq. $HgCl_2$	2600	0·440	0·360	0·86
4% Aq. $HgCl_2$	2700	0·105	0·125	0·23
Pyrex	2950	—	—	—
0·025% Aq. quinine HCl	3300	—	—	—
0·1% Aq. quinine HCl	3450	—	—	—
0·5% Aq. quinine HCl	3600	—	—	—

Experiments with Gelatin.—In view of the considerable similarities in general structure of the proteins to the amides, it was considered of interest to see whether any corresponding similarity existed in the manner of solution photolysis of the two types of compound. Gelatin was selected as a convenient material for examination. It is, unfortunately, completely insoluble both in hexane and in dioxan. Preliminary studies were made on a 1% aqueous solution. A disadvantage appears at once in that water is hardly a solvent suited to the ready hydrogenation of radicals. Furthermore, it is to be expected that the protein will undergo some hydrolytic decomposition in the course of a run, thereby complicating an already complex system. The possibility of assessing unsaturation, however or wherever it may arise, is also excluded, owing to a complex and irreproducible reaction between the protein and bromine.

Some figures for gas analysis of products drawn off at liquid-nitrogen temperature are given below in the table. It must be emphasised that some of the values given for hydrogen represent amounts tending towards the limit of accuracy of the analytical technique. It is believed, however, that they are significant.

Temp.	Time of photolysis (hours)	CO (ml.)	H_2 (ml.)	Temp.	Time of photolysis (hours)	CO (ml.)	H_2 (ml.)
30°	18	0·645	0·060	40°	14	0·680	0·060
30	17	0·215	0·020	40	24	1·035	0·025
40	14	0·685	0·090				

Discussion

Ammonia and Amines.—The main points that emerge from the results and require discussion can be summarised as follows: (*a*) The only permanent gaseous product of photodecomposition in hydrocarbon solution is hydrogen, except for a small amount of methane from ethylmethylamine. (*b*) The amount of unsaturation developed in the hydrocarbon solvent is, in general, equivalent to the amount of hydrogen evolved, provided that the absorbing substrate is present in low concentration. (*c*) At higher concentrations, the amount of unsaturation falls off with respect to the hydrogen yield. (*d*) An insoluble nitrogenous material is found in association with this loss of unsaturation. (*e*) The extent of the imbalance of unsaturation and hydrogen is both temperature- and time-dependent for a given concentration of amine. (*f*) At no time does the unsaturation in the liquid significantly exceed the hydrogen produced.

Most of these points may be covered if we suppose that the primary step in the photochemical process involves the removal of a hydrogen atom from the $-NH_2$ group:

$$R{\cdot}NH_2 + h\nu \longrightarrow R{\cdot}NH + H$$

When the amine (or ammonia, for which R above is replaced by H) is present in low concentration, the following set of dark reactions can then proceed:

$$H + C_6H_{14} \longrightarrow H_2 + C_6H_{13}$$
$$R{\cdot}NH + C_6H_{14} \longrightarrow R{\cdot}NH_2 + C_6H_{13}$$
$$2C_6H_{13} \longrightarrow C_6H_{12} + C_6H_{14}$$

and the amount of olefin, C_6H_{12}, produced corresponds with the amount of gaseous hydrogen formed. This primary step corresponds with that proposed by Bamford (*J.*, 1939, 17) for the photolysis of primary aliphatic amines in the gas phase.

An exactly similar scheme can be formulated for the secondary amine, ethylmethylamine, if we include a small contribution due to an initial step:

$$C_2H_5{\cdot}NH{\cdot}CH_3 \longrightarrow C_2H_5{\cdot}NH + CH_3$$

to account for the small amount of methane formed. No corresponding step is indicated in which an ethyl radical is split off since no ethane was detected in the products.

In the photodecomposition of methylamine and diethylamine in the gas phase, as reported by Bamford (*loc. cit.*) little methane was found, whereas Emeléus and Jolley (*J.*, 1935, 929) found large quantities of this gas in the thermal decomposition products. The small, but definite, amount of methane found in the present work suggests some slight overlap between the two types of decomposition.

The fact that the unsaturation never significantly exceeded the hydrogen precludes any possibility of reaction analogous to the Type II decomposition of the aldehydes and ketones of the form:

$$CH_3{\cdot}CH_2{\cdot}CH_2{\cdot}CH_2{\cdot}CH_2{\cdot}CH_2{\cdot}NH_2 \longrightarrow CH_3{\cdot}CH_2{\cdot}CH_2{\cdot}CH{:}CH_2 + CH_3{\cdot}NH_2$$

as this would clearly lead to an extra degree of unsaturation which, under the conditions of our experiments, would remain in the liquid.

As we increase the amine concentration, we introduce the probability of termination reaction in which the C_6H_{13} radicals, instead of undergoing hydrogen-exchange reactions with the solvent until two come together and disproportionate, may be hydrogenated at the expense of amine molecules. It has been shown by Trotman-Dickenson and Steacie (*J. Chem. Physics*, 1951, **19**, 329) that, for reactions of methyl radicals, the activation energies for hydrogen abstraction from paraffins and from amines differ extremely slightly. Hence at higher concentrations of amine, we may envisage reactions of the type:

$$C_6H_{13}{\cdot}NH_2 + C_6H_{13} \longrightarrow C_6H_{14} + C_6H_{13}{\cdot}NH$$

and

$$C_6H_{13}{\cdot}NH_2 + C_6H_{13} \longrightarrow C_6H_{14} + C_6H_{12}{\cdot}NH_2$$

It is clear that the former would not affect the issue, simply returning us to one stage in the simple scheme previously expounded. The second type, however, gives the likelihood of an ultimate compound of the type $C_6H_{11}\cdot NH_2$, in which the unsaturation has gone into the hydrocarbon chain of the amine. This unsaturation would be lost as far as determination by our experimental method is concerned, the unsaturated amine being removed in the acid wash.

Franck and Rabinowitch's principle (*Trans. Faraday Soc.*, 1934, **30**, 120) states that radicals produced by the fission of a chemical bond in solution are held together by a cage of solvent molecules and may make two or three collisons with each other before escaping. Under such conditions, primary recombination plays a large part in the ultimate fate of the radicals and the general low quantum yield of photochemical reactions in solution is explained. It seems quite probable, that, from this principle, an alternative to primary recombination will exist. To use the case of hexylamine as a specific example, hydrogenation of the hydrogen atom by the other fragment of the parent molecule, *i.e.*, the hexylamino-radical is to be expected. We would expect this to increase in probability as the temperature is raised.

Recent work in the gas-phase photolysis, by Johnson and Taylor (*loc. cit.*), suggests, as alternative to "lost" unsaturation, "extra" hydrogen arising from the thermal decomposition of the by-product polymer thus:

$$2CH_3\cdot NH_2 \longrightarrow H_2 + \frac{1}{n}(C_2H_8N_2)_n \text{ (over-all reaction)}$$

$$\frac{1}{n}(C_2H_8N_2)_n \longrightarrow H_2 + \frac{1}{n}(C_2H_6N_2)_n$$

$$\frac{1}{n}(C_2H_6N_2)_n \longrightarrow NH_3 + \frac{1}{n}(C_2H_3N)_n$$

They assume that the polymer of $C_2H_8N_2$ first arises by isomerisation of $CH_3\cdot NH$ radicals to $CH_2\cdot NH_2$ radicals, which then polymerise. We have indicated above how $C_6H_{12}\cdot NH_2$ radicals might arise directly without isomerisation. As no polymer was obtained at low initial amine concentration, this seems preferable to isomerisation.

We have been able to demonstrate a further evolution of hydrogen, after the lamp had been turned off, from a system favourable to the production of the polymer. No ammonia could be detected, however.

The observed fact that the fraction of "lost" unsaturation increases with time, under conditions of constant temperature and concentration, is in accord with the ideas of Johnson and Taylor (*loc. cit.*). It would seem that something formed in the course of the reaction is affecting its subsequent behaviour. Two types of compound are built up in the liquid phase during the reaction, namely olefin and the nitrogenous "sludge." It has been found that if we perform the irradiation in unpurified hexane solution, *i.e.*, hexane already containing an appreciable concentration of unsaturated constituents, the value of the ratio, unsaturation : hydrogen, after a given time, is the same as that obtained in the purified solvent. There is, of course, a reduction in the overall amount of decomposition owing to absorption of the active radiation by the solvent. The unsaturation loss (or hydrogen gain) therefore must be accounted for by some means such as that indicated above. A fuller knowledge of the precise nature of this polymeric material would be of very great value.

Amides.—The main points to be extracted from the results, on which a discussion may be formulated, are as follows: (*a*) Carbon monoxide and hydrogen are the gaseous products common to all the amides examined. (*b*) In all cases, primary amines are formed during the course of the reaction. (*c*) No methane is obtained from acetamide. (*d*) From propionamide, butyramide, valeramide, and hexanoamide we obtain some unsaturated hydrocarbon gas, this being established as ethylene from propionamide and butyramide and inferred as propylene and butylene from valeramide and hexanoamide. (*e*) Stearamide gives more liquid unsaturation than can be accounted for by the hydrogen yield. (*f*) Examination of end-products for the presence of aldehydes and αβ-diketones gives

negative results. (*g*) When colour filters are introduced, it is found that the ratio of carbon monoxide to hydrogen increases with increase in wave-length.

We can account for these facts if we suppose that there are, in general, two different modes of decomposition for the amides, which are completely analogous respectively to the Type I decomposition of the aldehydes and Type II decomposition for the aldehydes and ketones as propounded by Bamford and Norrish (*loc. cit.*).

These processes may be represented by the following equations, butyramide being used as a typical example :

$$CH_3{\cdot}CH_2{\cdot}CH_2{\cdot}CO{\cdot}NH_2 + h\nu \longrightarrow CH_3{\cdot}CH_2{\cdot}CH_2{\cdot}NH_2 + CO \quad \text{Type I}$$
$$CH_3{\cdot}CH_2{\cdot}CH_2{\cdot}CO{\cdot}NH_2 + h\nu \longrightarrow CH_2{:}CH_2 + CH_3{\cdot}CO{\cdot}NH_2 \quad \text{Type II}$$

We then have the reactions :

$$CH_3{\cdot}CH_2{\cdot}CH_2{\cdot}NH_2 + h\nu \longrightarrow CH_3{\cdot}CH_2{\cdot}CH_2{\cdot}NH + H, \text{ etc., as for amines}$$
$$CH_3{\cdot}CO{\cdot}NH_2 + h\nu \longrightarrow CH_3{\cdot}NH_2 + CO \quad \text{Type I}$$
$$CH_3{\cdot}NH_2 + h\nu \longrightarrow CH_3{\cdot}NH + H, \text{ etc., as for amines}$$

On a scheme such as this, the hydrogen yield will equal the amount of unsaturation not accounted for by the Type II reaction. This is found in fact to be the case for butyramide and valeramide, where the ethylene and propylene respectively could be completely separated from solution in the hydrocarbon solvent.

For acetamide, no Type II decomposition is possible and the reaction is simply of Type I. The complete absence of methane from all the reactions, but in particular from the acetamide system, indicates that the Type I reaction is of the aldehyde type, *i.e.* :

$$R{\cdot}CO{\cdot}NH_2 \longrightarrow R{\cdot}NH_2 + CO$$

and not of the ketone type :

$$R{\cdot}CO{\cdot}NH_2 \longrightarrow R + CO{\cdot}NH_2 \longrightarrow R + CO + NH_2$$

or

$$R{\cdot}CO{\cdot}NH_2 \longrightarrow R{\cdot}CO + NH_2 \longrightarrow R + CO + NH_2$$

since the latter would give subsequent reactions such as (for acetamide) :

$$CH_3 + C_6H_{14} \longrightarrow CH_4 + C_6H_{13}$$
$$CH_3{\cdot}CO + C_6H_{14} \longrightarrow CH_3{\cdot}CHO + C_6H_{13}$$
$$NH_2 + C_6H_{14} \longrightarrow NH_3 + C_6H_{13}, \text{ etc.}$$

and perhaps

$$2CH_3{\cdot}CO \longrightarrow CH_3{\cdot}CO{\cdot}CO{\cdot}CH_3$$

and we should expect materials in the final mixture which have been shown to be absent.

We are thus led to the conclusion that, in Type I reactions, we have decomposition into molecules rather than into free radicals, *i.e.*, an aldehyde Type I reaction.

Owing to the extremely low solubility of the amides in hydrocarbons, the subsequent amine concentration is also very low. Thus, the amine photolysis will be uncomplicated by the concentration effects observed for these compounds, and the observed balance between hydrogen yield and liquid unsaturation (apart from Type II) might be expected. The observed lack of coloration of the solvent and separation of polymeric material during the subsequent handling is in accord with these ideas.

The general indication from the treatment above is that it is either the C=O group or the complete $CO{\cdot}NH_2$ group rather than the bare NH_2 group which controls the decomposition of amides. This is in accord with expectation from consideration of the absorption spectra of these substances. Bielecki and Henri (*Compt. rend.*, 1913, **156,** 1860), in measurements of the molar extinction coefficients in alcoholic solution, give values which may be compared thus :

	λ	ε		λ	ε
$CH_3{\cdot}NH_2$	2405	0·54	$CH_3{\cdot}CH_2{\cdot}NH_2$	2525	0·22
$CH_3{\cdot}CO{\cdot}NH_2$	2405	8·1	$CH_3{\cdot}CH_2{\cdot}CO{\cdot}NH_2$	2525	1·01

showing a large increase in absorption at a given wave-length by the introduction of the C=O group into the molecule. If the production of hydrogen arises from the photolysis of amines produced after Type I decomposition of the amide as we have suggested, then, by increasing the wave-length available for absorption, we should reduce the amount of hydrogen produced with respect to the carbon monoxide. An inspection of the table (p. 194) showing the effect of filters bears out this contention. We would expect no such reduction in the hydrogen yield, if, after absorption of a quantum of energy by the C=O group, the molecule were to decompose by a mechanism such as :

$$R{\cdot}CO{\cdot}NH_2 + h\nu \longrightarrow R{\cdot}CO{\cdot}NH_2^* \longrightarrow R{\cdot}CO{\cdot}NH + H$$

A comparison of the results on stearamide in hydrocarbon at 40° and 100° shows a marked increase in the hydrogen : carbon monoxide ratio at the higher temperature, and little change in the absolute amount of carbon monoxide. This is in accord with the idea that the carbon monoxide arises from a primary molecular photochemical step and that the hydrogen comes from a process requiring activation as has been postulated.

A difficulty appears in the case of propionamide. Here, Type II decomposition would give :

$$CH_3{\cdot}CH_2{\cdot}CO{\cdot}NH_2 \longrightarrow CH_2 + CH_3{\cdot}CO{\cdot}NH_2$$

and presumably the methylene would ultimately appear as methane. In practice we get ethylene. Bamford and Norrish (*loc. cit.*) found a similar phenomenon in the photolysis of ethyl methyl ketone which gave ethylene and acetaldehyde. They distinguished this reaction from the usual (Type II) reaction involving a fission of the bond between the α- and the β-carbon atoms by calling it Type III. In the case of propionamide, this would give :

$$CH_3{\cdot}CH_2{\cdot}CO{\cdot}NH_2 \longrightarrow CH_2{:}CH_2 + H{\cdot}CO{\cdot}NH_2$$

in addition to the general Type I reaction.

Protein.—The results on gelatin show that some at least of the photochemical degradation occurs in a manner similar to amide photolysis. It is quite clear that this is by no means the whole story, as is shown by the extensive work of Mandl and McLaren (*Nature*, 1949, **164,** 750; *J. Amer. Chem. Soc.*, 1951, **73**, 1826), and Roberts and Rideal (*Proc. Roy. Soc., A*, 1951, **205**, 391), who have demonstrated the occurrence of deamination and further condensation reactions.

One of us (G. H. B.) thanks the British Rayon Research Association for the award of a Research Scholarship, during the tenure of which most of this investigation was carried out, and the Anglo-Iranian Oil Company for a subsidiary grant.

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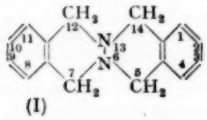
40. *Heterocyclic Nitrogen Compounds. Part II.* The Preparation of 5 : 7 : 12 : 14-Tetrahydro-6 : 13-diazanaphthacene and Some Derivatives thereof, with an Example of Ring Expansion and Contraction in a Clemmensen Reduction.*

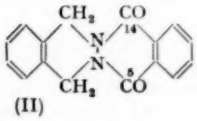
By H. H. Hatt and (Miss) E. F. M. Stephenson.

Condensation of tetrahydrophthalazine hydrochloride with ωω′-dibromo-*o*-xylene (*o*-xylylene dibromide) gives 5 : 7 : 12 : 14-tetrahydro-6 : 13-diazanaphthacene (I),* and with *o*-bromomethylbenzoyl bromide gives the 5-keto-derivative of (I). Clemmensen reduction of the 5 : 14-diketo-compound (II) proceeds with molecular rearrangement and formation, first, of the tetracyclo-compound (III) containing a 7–5-ring system. Continued reduction leads by hydrogenolysis of the 7-membered ring to the primary amine (IV), the structure of which is established by conversion into *N*-*o*-xylylphthalimidine. The mechanism of the Clemmensen reduction is discussed.

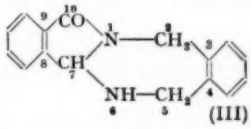
In continuation of the investigation of diazanaphthacene derivatives reported in Part I,* the parent 5 : 7 : 12 : 14-tetrahydro-6 : 13-diazanaphthacene (I) has been prepared by heating together tetrahydrophthalazine hydrochloride and ωω′-dibromo-*o*-xylene (*o*-xylylene dibromide). The 5-keto-derivative of (I) has been prepared similarly from tetrahydrophthalazine hydrochloride and *o*-bromomethylbenzoyl bromide.



(I)

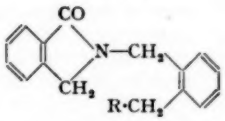


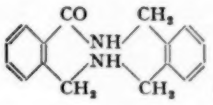
(II)



(III)

(IV; R = NH_2)
(V; R = OH)
(VI; R = Cl)
(VII; R = H)





(VIII)

The tetrahydrodiazanaphthacene (I) is basic and forms well-defined salts, in contrast to the related tetrabenzylhydrazine (Wieland and Schamberg, *Ber.*, 1920, **53**, 1329). Its stability also is noteworthy : it is unaffected by most hydrolytic and reducing agents, for instance, zinc and concentrated hydrochloric acid, under conditions which suffice to sever the nitrogen–nitrogen bond of phthalazine.

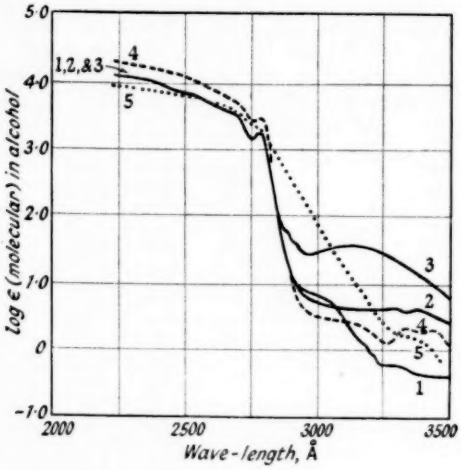
The above syntheses are not very convenient, since tetrahydrophthalazine is not readily prepared. Reduction of the more accessible 5 : 14-diketo-compound (II) (Part I) could provide a more convenient method, but (II) is resistant to most reducing agents, and the Clemmensen reagent, which does reduce it, severs the nitrogen–nitrogen bond. Catalytic methods of reduction have yet to be examined. The chief products of the Clemmensen reduction of (II) were two compounds which have been assigned structures (III) and (IV). In addition, some ωω′-diamino-*o*-xylene was always formed, although usually in small quantity. If the amount of hydrochloric acid used was restricted, or if the reaction time was short, 10-keto-1 : 6-diaza-3 : 4-8 : 9-dibenzo*bicyclo*[5 : 3 : 0]deca-3 : 8-diene (III) was the major product; otherwise (IV) predominated, and with long reaction times (III) disappeared, being reduced to (IV) although not quantitatively.

Compound (III) is monoacidic, forms monoacyl and mononitroso-derivatives, and can be recovered from the last by hydrolysis. Concentrated hydrochloric acid hydrolyses (III) to ωω′-diamino-*o*-xylene and phthalaldehydic acid (isolated as 3-ethoxyphthalide).

* Part I, *J.*, 1943, 658, where (I) was named on the Ring Index system (*i.e.*, as a tetrahydro-derivative of diazanaphthacene rather than as a diaza-derivative of hexahydronaphthacene) but numbered on an arbitrary system. In the present paper, the Ring Index names are retained but the numbering is changed to that required by the Ring Index system.

These facts establish its structure and explain the failure to reduce it quantitatively to (IV). The infra-red spectrum also agrees with structure (III). Absorption bands appear at 1624 cm.$^{-1}$ (NH deformation), 1683 cm.$^{-1}$ (C=O stretching), and 3280 cm.$^{-1}$ (NH stretching). The 1624 cm.$^{-1}$ band could be ascribed to C=N stretching, but occurs also in the spectrum of phthalimidine, which shows no OH band and can therefore show no bands attributable to C=N.

N-o-Aminomethylbenzylphthalimidine (IV) forms a monohydrochloride, but, unlike (III), is otherwise unaffected by hydrochloric acid or by the Clemmensen reagent. Its structure follows from its conversion by nitrous acid into the corresponding primary alcohol (V), thence with hydrochloric acid into the chloromethyl compound (VI), and finally by reduction of this with zinc and acetic acid into *N-o*-xylylphthalimidine (VII), identical with the reduction product of *N-o*-xylylphthalimide. In agreement with structure (IV), the product from the Clemmensen reduction forms mono-benzoyl, -toluene-*p*-sulphonyl, and -2 : 4-dinitrophenyl derivatives, and gives a benzylidene derivative under acid catalysis. A comparison of the ultra-violet absorption spectra of (III), (IV), (V), (VII), and *N*-benzylphthalimidine also gives support to structure (IV) (see figure). The absorption spectrum



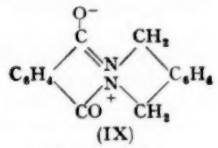
(1) N-o-*Xylylphthalimidine.*
(2) N-o-*Aminomethylbenzylphthalimidine.*
(3) N-o-*Hydroxymethylbenzylphthalimidine.*
(4) *N-Benzylphthalimidine.*
(5) 10-*Keto*-1 : 6-*diaza*-3 : 4-8 : 9-*dibenzo*-bicyclo[5 : 3 : 0]*deca*-3 : 8-*diene.*

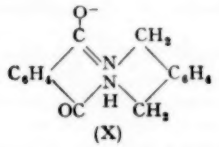
of (III) differs noticeably from the others, which strongly resemble one another. These last all exhibit a characteristic small band at 2780 Å, which for *N*-benzylphthalimidine has a molecular extinction coefficient of 2650. The other three compounds, (IV), (V), and (VII), possess almost identical molecular extinction coefficients at this wave-length : 1560, 1590, and 1550, respectively, agreeing with their similar structures. The infra-red spectrum of (IV) in a Nujol suspension shows absorption bands at 1622 cm.$^{-1}$ (NH deformation), weak 1639 cm.$^{-1}$ (C=N stretching?), 1683 cm.$^{-1}$ (C=O stretching), and at 3295 and 3370 cm.$^{-1}$ (NH stretching).

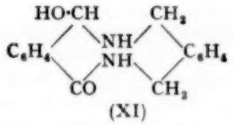
Some difficulties, however, arise in accepting formula (IV) as the sole structure. The arylsulphonyl derivatives are insoluble in alkali, and the compound is unstable to alcoholic sodium ethoxide towards which (V) and other *N*-substituted phthalimidines are stable. The compound also gives a diacetyl derivative of unexpected stability, for although it can be hydrolysed again to the parent base, it has not yet been hydrolysed to a monoacetyl derivative. These properties may mean that (IV) can undergo some isomerisation to the ten-ring structure (VIII).

The reduction of (II) to (III) is an example of a molecular rearrangement during the course of a Clemmensen reduction which involves ring expansion and contraction. It resembles the rearrangements studied by Prelog, Clemo, Leonard, and their respective co-workers (Prelog and Seiwerth, *Ber.*, 1939, **72**, 1638; Clemo, Raper, and Vipond, *J.*, 1949, 2095; Clemo and Vipond, *Chem. and Ind.*, 1949, 856; Leonard and Wildman, *J. Amer.*

Chem. Soc., **1949**, **71**, 3089; Leonard and Ruyle, *ibid.*, p. 3094). It differs from them in that a nitrogen–nitrogen, not a carbon–nitrogen bond, unites positions 2 and 3 relative to the carbonyl group, and suffers fission during reduction. In this type of reduction we consider the first step to be an attack by electrons from the metal on the canonical form (IX), leading to (X) by subsequent addition of a proton, and thence to the amino-alcohol (XI) from which (III) arises by cyclisation.


(IX)


(X)


(XI)

Comparison with the behaviour of (I) shows that the carbonyl group in (II) clearly promotes hydrogenolysis of the 2 : 3-bond. In a similar manner it can be regarded as promoting hydrogenolysis of the carbon–nitrogen bond in (III), so that, although (III) is readily hydrolysed by acid to phthalaldehydic acid and ωω′-diamino-*o*-xylene, yet it can be reduced to (IV) in good yield by the Clemmensen reagent. On this explanation (III) should first be reduced to (VIII) and pass to (IV) by isomerisation.

Experimental

M.p.s are corrected. Micro-analyses were by Mr. H. L. Oates, Miss E. E. Rutherford, and by the authors.

5 : 7 : 12 : 14-*Tetrahydro*-6 : 13-*diazanaphthacene* (I).—Equimolecular quantities of dry, finely powdered 1 : 2 : 3 : 4-tetrahydrophthalazine hydrochloride (6·45 g.) and ωω′-dibromo-*o*-xylene (10 g.) were heated for 1 hour at 140—150° in the absence of moisture. After cooling, the product was ground with a little alcohol to remove tarry impurities, and the crystalline mixture of the hydrochloride and hydrobromide of the base (6·5 g.) was converted into the free base by excess of 5% aqueous sodium hydroxide. From 40% aqueous alcohol (charcoal), the free *base* crystallised in thin colourless plates, m. p. 132—133° (3·6 g.) (Found : C, 81·5; H, 6·6; N, 11·8. $C_{16}H_{16}N_2$ requires C, 81·3; H, 6·8; N, 11·9%). Gradual addition of the tetrahydrophthalazine hydrochloride to the molten dibromide did not improve this yield. Condensation did not take place in pyridine; in boiling xylene condensation occurred, but the yield was poor. The *hydrochloride* was obtained as colourless needles (from absolute alcohol–light petroleum), m. p. 248—250° (decomp.) (Found : C, 70·5; H, 6·2; N, 10·7; Cl, 13·1. $C_{16}H_{17}N_2Cl$ requires C, 70·5; H, 6·3; N, 10·3; Cl, 13·0%). The *methiodide* separated as pale yellow parallelepipeds on addition of excess of methyl iodide to the base; it had m. p. 221—223° (decomp.), dependent on the rate of heating (Found : I, 33·8. $C_{17}H_{19}N_2I$ requires I, 33·6%).

The base could be neither acetylated nor benzoylated. It was unaffected by alcoholic sodium ethoxide, sodium dithionite (hydrosulphite), fuming hydriodic acid, stannous chloride with alcoholic hydrogen chloride, or zinc and hydrochloric acid. It gave a colourless solution in concentrated sulphuric acid. Nitrous acid caused decomposition to unidentified products.

Oxidation of the base with potassium permanganate in acetone produced only small amounts of phthalazine, probably because under the conditions needed to attack (I) it also was attacked. From 1·0 g. of (I), 0·06 g. of phthalazine was obtained, m. p. 143·5—144·5° (identified by conversion into the methiodide and picrate and by mixed m. p.). Phthalic acid was the only other product identified.

5 : 7 : 12 : 14-*Tetrahydro*-5-*keto*-6 : 13-*diazanaphthacene*.—Finely powdered, dry tetrahydrophthalazine hydrochloride (3·0 g.) and *o*-bromomethylbenzoyl bromide (5·4 g.) were mixed and heated at 150—160°, with exclusion of moisture. After the vigorous frothing and evolution of gas had ceased (30 minutes) the temperature was increased to 200—210° and heating continued (30 minutes). The red resinous product, when cool, was triturated with a little methanol, the crystalline material was washed with a little methanol, and basic materials were removed by digestion with warm dilute hydrochloric acid. The insoluble *ketone* was dissolved in benzene, freed from coloured impurities by chromatography on alumina, and recovered from the benzene eluate. It crystallised from methanol in colourless crystals, m. p. 198—199° (decomp.) (0·7 g.) (Found : C, 76·8; H, 5·5; N, 11·3. $C_{16}H_{14}ON_2$ requires C, 76·8; H, 5·6; N, 11·2%).

Clemmensen Reduction of 5 : 7 : 12 : 14-*Tetrahydro*-5 : 14-*diketo*-6 : 13-*diazanaphthacene* (II).—The small solubility of (II) made it necessary to use alcohol as a solvent. The amounts of compounds (III) and (IV) produced from (II) depended on the amount of hydrochloric acid used, its rate of addition, and the time of reaction. From (II) with 5 parts of zinc and 20 parts of hydrochloric acid a 20—25% yield of (III) was obtained in a reaction time of 20 hours. With twice this amount of hydrochloric acid and a reaction time of 30 hours, (IV) was the main product (yield, 55—60%) and (III) was either absent or present in small amount. However, it was difficult to duplicate yields closely. In the example given below, compound (IV) is the the major product.

Amalgamated zinc (70 g.), prepared from pure zinc (B.D.H. arsenic-free), ethanol (200 ml.), concentrated hydrochloric acid (100 ml.), and 5 : 7 : 12 : 14-tetrahydro-5 : 14-diketo-6 : 13-diazanaphthacene (13·2 g., 0·05 mole) were refluxed together for 30 hours. Additional hydrochloric acid was added at 3-hourly intervals (total, 120 ml.). After the unchanged zinc had been removed, the liquors were concentrated to half bulk, a further 40 ml. of concentrated acid was added, and after 24 hours the crystalline zincichlorides were collected (A). The filtrates were made strongly alkaline with 30% aqueous sodium hydroxide and cooled, and the precipitated bases were collected by centrifuging after 24 hours (B). The aqueous layers were usually discarded, but ωω′-diamino-*o*-xylene could be slowly distilled from them in steam and was isolated as the hydrochloride (m. p. 310°, decomp.) and identified by conversion into the dibenzoyl derivative (m. p. and mixed m. p. 189—190°). Gabriel and Pinkus (*Ber.*, 1893, **26**, 2213) reported m. p. 184°.

The zincichlorides (A) were decomposed by dissolving them in 250 ml. of 50% aqueous acetic acid, liberating the bases as oils with excess of 30% aqueous sodium hydroxide, and collecting them when solidified. From this material and (B), compounds (III) and (IV) were separated by taking advantage of their different solubilities either in alcohol [1 g. of (III) dissolves in 100 ml. of hot alcohol; 1 g. of (IV) in 2 ml.] or in aqueous acetic acid, in which only the stronger base (IV) dissolved. This experiment yielded 1·4 g. of (III) and 7·0 g. of (IV). It is important to use pure zinc in the Clemmensen reduction. Some samples of zinc used contained traces of sulphur, which was reduced to hydrogen sulphide and this, with compound (IV), gave a *substance*, m. p. 222—223° (Found : C, 72·6; H, 5·6; N, 10·2; S, 6·0. $C_{32}H_{32}O_2N_4S$ requires C, 71·6; H, 6·0; N, 10·4; S, 6·0%). This compound was readily separated from (III) and (IV), being only slightly soluble in boiling dilute aqueous acetic acid. It crystallised well from butanol. Its structure is being investigated.

10-*Keto*-1 : 6-*diaza*-3 : 4-8 : 9-*dibenzo*bicyclo[5 : 3 : 0]*deca*-3 : 8-*diene* (III). From ethanol, benzene, or ethyl acetate this compound separated in fine colourless needles, m. p. 254—255° (decomp.). It sublimed unchanged at 190—200°/1 mm. [Found : C, 77·0; H, 5·8; N, 11·3; *M* (in camphor), 251. $C_{16}H_{14}ON_2$ requires C, 76·8; H, 5·6; N, 11·2%; *M*, 250]. Hydrochloric acid dissolved (III), and with immediate cooling the *hydrochloride* separated. Recrystallised from ethanol, it formed colourless needles (m. p. 273—276°, decomp.) containing one molecule of water of crystallisation (Found : N, 9·1; loss at 110°, 5·6. $C_{16}H_{15}ON_2Cl,H_2O$ requires N, 9·1; H_2O, 5·9. Found, in dried salt : N, 9·8; Cl, 11·9. $C_{16}H_{15}ON_2Cl$ requires N, 9·7; Cl, 12·3%). The ketone (III) was stable in boiling alcoholic potassium hydroxide. The *benzoyl* derivative, readily obtained under Schotten–Baumann conditions, formed from ethanol colourless rhombs (m. p. 168·5—169·5°) (Found : C, 78·3; H, 5·4; N, 7·9. $C_{23}H_{16}O_2N_2$ requires C, 78·0; H, 5·1; N, 7·9%). An *acetyl* derivative was formed in boiling acetic anhydride and precipitated with water; it formed colourless needles (from 40% methanol), m. p. 193·5—194° (Found : C, 74·0; H, 5·4; N, 9·6. $C_{18}H_{16}O_2N_2$ requires C, 74·0; H, 5·5; N, 9·6%). A toluene-*p*-sulphonyl derivative could not be obtained. The *nitroso*-derivative was obtained by cooling a 3% solution of (III) in glacial acetic acid to 10° and adding the theoretical amount of sodium nitrite in twice its weight of water. After 2 hours, dilution with water precipitated the nitroso-compound in nearly theoretical yield. It crystallised from chloroform and ether in pale yellow, thin, hexagonal plates, or from *n*-butanol in stout prisms, m. p. 204° (Found : C, 69·3; H, 4·7; N, 15·0. $C_{16}H_{13}O_2N_3$ requires C, 68·8; H, 4·7; N, 15·0%). This compound gave the Liebermann nitroso-reaction. Short hydrolysis with either hot hydrochloric acid or 10% alcoholic sodium hydroxide yielded (III), together with some oily by-products.

Hydrolysis of (III).—Complete hydrolysis was achieved by refluxing for 12 hours with 6N-hydrochloric acid. The acid was then removed by evaporation under reduced pressure and desiccation completed by addition of alcohol containing a little hydrochloric acid, evaporation, and repeating this process once. The crystalline ωω′-diamino-*o*-xylene dihydrochloride hemihydrate was then separated from the oily product by addition of ethanol containing a drop of

hydrochloric acid, and collection and washing of the crystals with a little ethanol; the yield, from 3 g. of (III), was 2·5 g. (theory, 2·62 g.). The hydrochloride [m. p. 310° (decomp.)] was identified by conversion into the dibenzoyl derivative (m. p. and mixed m. p. 189—190°) and the dibenzenesulphonyl derivative (m. p. and mixed m. p. 125—126°). The alcoholic solution of the oily product on evaporation gradually gave crystalline 3-ethoxyphthalide (yield, 1·7 g.; theory, 2·1 g.), which had m. p. 69—70° after crystallisation from light petroleum containing 3% of ethanol. It was identified by mixed m. p. and by conversion into phthalazone and the anil of phthalaldehydic acid. These two compounds are as conveniently prepared from 3-ethoxyphthalide as from phthalaldehydic acid.

Phthalazone. 3-Ethoxyphthalide (0·36 g.), hydrazine sulphate (0·26 g.), sodium acetate (0·6 g.), and water (25 ml.) were heated under reflux (1 hour). From the solution, decolorised with a little charcoal, colourless needles of phthalazone (0·16 g.) separated on cooling (m. p. and mixed m. p. 182—183°).

Anil of phthalaldehydic acid. The anil was precipitated in crystalline form (yield 0·11 g.) when 3-ethoxyphthalide (0·1 g.), aniline (0·1 ml.), acetic acid (0·15 ml.), and water (12 ml.) were refluxed for 5 minutes (m. p. and mixed m. p. 179—180° after crystallisation from ethanol).

The following new derivatives of ωω′-diamino-*o*-xylene were prepared: *ditoluene*-p-*sulphonyl* (Schotten–Baumann), colourless prisms (from 50% methanol), m. p. 122·5—123·5° (Found: C, 59·8; H, 5·4; N, 6·0. $C_{22}H_{24}O_4N_2S_2$ requires C, 59·4; H, 5·4; N, 6·3%); *dipicrate* (from water), m. p. 242—244° (decomp.) (Found: C, 40·6; H, 3·2; N, 19·0. $C_{20}H_{16}O_{14}N_8$ requires C, 40·4; H, 3·1; N, 18·9%).

N-o-*Aminomethylbenzylphthalimidine* (IV).—This compound is dimorphous. It crystallised from benzene or benzene–ether in needles, m. p. 108·5—109°, or in stout rhombs, m. p. 120—121°. Both were pale cream in colour. By inoculation either form could be obtained at will and, in absence of the high-melting form, the less stable lower-melting form kept indefinitely [Found: C, 76·3; H, 6·3; N, 11·1%; *M* (in camphor), 237. $C_{16}H_{16}ON_2$ requires C, 76·2; H, 6·4; N, 11·1%; *M*, 252]. The *amine* was slightly soluble in water, readily soluble in ethanol, benzene, and chloroform, and almost insoluble in ether or light petroleum. It showed a strong blue fluorescence in ultra-violet light. It had a bitter taste. Although the two forms of the base appeared to be dimorphs, differing but little in stability, it is noteworthy that on benzoylation they gave corresponding dimorphous forms of the benzoyl derivative. The amine (IV) gave a salt with hydriodic acid, but otherwise was unaffected. It was gradually attacked by N-alcoholic potassium hydroxide, but the products of decomposition have not been identified. It decomposed slowly above its m. p.

The *hydrochloride* crystallised from ethanol in fine white needles, m. p. 243—245° (Found: C, 67·0; H, 5·9; N, 10·3; Cl, 12·6. $C_{16}H_{17}ON_2Cl$ requires C, 66·6; H, 5·9; N, 9·7; Cl, 12·3%). The *hydriodide* formed pale yellow needles (from ethanol), m. p. 236—238° (decomp.) (Found: C, 50·6; H, 4·7; N, 7·3. $C_{16}H_{17}ON_2I$ requires C, 50·5; H, 4·5; N, 7·4%). The *chloroaurate* formed bright yellow prisms (from ethanol), m. p. 224—226° (decomp.) (Found: C, 32·6; H, 3·0; N, 5·2; Au, 33·4. $C_{16}H_{17}ON_2Cl_4Au$ requires C, 32·4; H, 2·9; N, 4·7; Au, 33·3%).

The *diacetyl* derivative (m. p. 127·5—128·5°) was obtained by boiling (IV) with acetic anhydride (1 hour); from 40% ethanol, it separated in small colourless needles (Found: C, 70·6; H, 5·7; N, 8·3; Ac, 24·7. $C_{20}H_{20}O_3N_2$ requires C, 71·4; H, 6·0; N, 8·3; Ac, 25·6%). The diacetyl derivative was hydrolysed to the parent base (IV) by refluxing it (5 hours) with 6N-hydrochloric acid. Benzoylation of the low-melting form under Schotten–Baumann conditions, or in pyridine, gave a *benzoyl* derivative of m. p. 185°, and like treatment of the high-melting form gave first a benzoyl derivative of m. p. 197° but later the lower-melting material. From ethanol, the more soluble low-melting form separated in stout gable-ended prisms, and the high-melting form in fine white needles. The former was converted into the latter form at 188—190°, but conversion appeared to be incomplete and purification was effected by crystallisation from ethanol, leaving a few crystals undissolved. Conversion of the high- into the low-melting form resulted from prolonged boiling of an alcoholic solution to destroy all crystalline nuclei or by dissolution in chloroform and precipitation with light petroleum [Found: C, 77·4; H, 5·9; N, 7·9%; *M* (in camphor), 341. $C_{23}H_{20}O_2N_2$ requires C, 77·5; H, 5·7; N, 7·9%; *M*, 356]. Attempts to hydrolyse the benzoyl derivative failed.

The *benzenesulphonyl* derivative was prepared quantitatively from (IV) by using benzenesulphonyl chloride in pyridine at room temperature. From *n*-butanol (charcoal) it formed colourless prisms, m. p. 196·5—198° (Found: C, 67·6; H, 5·1; N, 6·6; S, 8·5. $C_{22}H_{20}O_3N_2S$ requires C, 67·3; H, 5·1; N, 7·1; S, 8·2%). The *toluene*-p-*sulphonyl* derivative, m. p. 173°, was obtained from either dimorph under Schotten–Baumann conditions, or in pyridine at room

temperature or at the b. p. It crystallised from methanol or chloroform–ether in stout rhombs and fluoresced a strong blue in ultra-violet light [Found : C, 67·7; H, 5·4; N, 6·6; S, 8·2%; *M* (in camphor), 415. $C_{23}H_{22}O_3N_2S$ requires C, 67·9; H, 5·5; N, 6·9; S, 7·9%; *M*, 406]. Both arylsulphonyl derivatives were insoluble in aqueous sodium hydroxide.

The 2 : 4-*dinitrophenyl* derivative was obtained by refluxing (2 hours) equimolecular amounts of (IV) and 2 : 4-dinitrochlorobenzene in ethanol solution with anhydrous sodium acetate and formed yellow needles, m. p. 205—207° (decomp.) from butanol (Found : C, 63·4; H, 4·3; N, 13·2. $C_{22}H_{18}O_5N_4$ requires C, 63·2; H, 4·3; N, 13·4%). The N-*phthaloyl* derivative was obtained by refluxing (1 hour) equimolar amounts of (IV) and phthalic acid in acetic acid, and precipitation with water. From butanol it crystallised in colourless, stout prisms, m. p. 206·5—208° (slight decomp.) (Found : C, 75·6; H, 4·6; N, 7·2. $C_{24}H_{18}O_3N_2$ requires C, 75·4; H, 4·7; N, 7·3%). The *benzylidene* derivative was obtained by refluxing (IV) for 1 hour with benzaldehyde (20% excess) in ethanol acidified with acetic acid. The product, obtained by removing the alcohol and making the solution alkaline, crystallised from benzene or 75% ethanol in colourless plates, m. p. 155·5—156·5° (slight decomp.) (Found : C, 81·0; H, 5·6; N, 8·1. $C_{23}H_{20}ON_2$ requires C, 81·2; H, 5·9; N, 8·2%). The *picrate*, obtained by mixing the components in alcohol and crystallisation from *n*-butanol, formed yellow needles, m. p. 240—241° (decomp.) (Found : C, 55·2; H, 3·8; N, 14·5. $C_{22}H_{19}O_8N_5$ requires C, 54·9; H, 4·0; N, 14·6%).

Clemmensen Reduction of (III).—A mixture of (III) (4·2 g.), amalgamated zinc (24 g.), ethanol (70 ml.), and concentrated hydrochloric acid (35 ml.) was refluxed (30 hours), and additional hydrochloric acid (75 ml.) added at 3-hourly intervals during the reaction. The products were worked up as already described, and 1·5 g. of unchanged (III) and 1·8 g. of (IV) were obtained. The latter melted at 117—119° and was identified by mixed m. p. and by conversion into the benzoyl derivative. No other products were isolated.

N-o-*Hydroxymethylbenzylphthalimidine* (V).—The reaction of (IV) with nitrous acid in acetic acid medium takes place readily at 0° or at room temperatures, but conversion into (V) is incomplete particularly at the lower temperature. The conversion can be completed by subsequently refluxing the reaction products with aqueous alkali. When the reaction was performed at 0° no crystalline (V) could be obtained without alkali treatment; at 20° some crystalline (V) could be obtained directly, but, always, refluxing with alkali improved the yield. Nitrogen is evolved during treatment with alkali and apparently the diazo-compound possesses appreciable stability under the reaction conditions used. The following method was adopted for the preparation of (V).

A solution of sodium nitrite (2·07 g., 0·03 mole) in water (9 ml.) was added during 5—10 minutes to a solution of (IV) (2·52 g., 0·01 mole) in acetic acid (20 ml.) and water (3 ml.) at 15—20° (cooling). After 12 hours the clear yellow solution was diluted with water (30 ml.), the separated oil extracted in benzene, the solution washed free from acid, the benzene removed on a water-bath, and the residue boiled (40 minutes) with 30% sodium hydroxide (70 ml.). The dark oil, after being washed free from alkali was dissolved in benzene, filtered from insoluble by-products, and concentrated to give a crystalline product (1·9 g.). From aqueous alcohol (charcoal), the *hydroxy*-compound formed colourless, stout prisms, m. p. 128·5—130° (Found : C, 76·2; H, 6·1; N, 5·7. $C_{16}H_{15}O_2N$ requires C, 75·9; H, 6·0; N, 5·5%). The infra-red absorption spectrum of (V) in chloroform shows C=O vibration at 1671 cm.$^{-1}$ and a strong OH vibration at 3400 cm.$^{-1}$. The compound is stable to boiling alcoholic N-sodium ethoxide. The 3 : 5-*dinitrobenzoyl* derivative, prepared in pyridine at room temperatures, crystallised from *n*-butanol (charcoal) in flat yellow prisms, m. p. 194·5—196·5° (decomp.) (Found : C, 61·9; H, 3·9; N, 9·0. $C_{23}H_{17}O_7N_3$ requires C, 61·7; H, 3·8; N, 9·4%). The *benzylidene* derivative was prepared by refluxing (2 hours) (V) (0·25 g.) in alcohol (20 ml.) with benzaldehyde (5 drops) and 40% sodium hydroxide (4 drops), removing the alcohol, and washing the residual oil with water. The dried product slowly crystallised. It was boiled with benzene (5 ml.) and the crystalline residue (0·12 g.) was recrystallised from aqueous alcohol. It formed pale cream needles, m. p. 198—199° (decomp.) (Found : C, 80·7; H, 5·6; N, 4·4. $C_{23}H_{19}O_2N$ requires C, 80·9; H, 5·6; N, 4·1%).

N-o-*Chloromethylbenzylphthalimidine* (VI).—A mixture of (V) (2·5 g.), concentrated hydrochloric acid (30 ml.), and water (20 ml.) was refluxed for 8—10 hours (oil-bath), cooled, and diluted with water. The oil produced was washed, and crystallised when dried under reduced pressure. From aqueous alcohol it formed colourless, blade-shaped plates (1·82 g.), m. p. 119·5—121° (Found : C, 71·0; H, 5·2; N, 5·3; Cl, 12·6. $C_{16}H_{14}ONCl$ requires C, 70·7; H, 5·2; N, 5·2; Cl, 13·0%). Refluxing of this compound with 10% aqueous sodium carbonate for 5 hours regenerated (V) almost quantitatively. Its β-*naphthylamine* derivative was prepared by re-

fluxing it (4 hours) in alcohol with sodium acetate and a small excess of β-naphthylamine. After precipitation with water and drying, the crystalline product was digested with benzene to dissolve impurities and crystallised from *n*-butanol; it formed colourless plates, m. p. 194·5—195·5° (Found: C, 82·4; H, 5·7; N, 7·2. $C_{26}H_{22}ON_2$ requires C, 82·5; H, 5·9; N, 7·4%).

N-o-*Xylylphthalimidine* (VII).—(*a*) *N-o*-Xylylphthalimide, prepared according to Strassmann (*Ber.*, 1888, **21**, 576), had m. p. 150·5—151·5° (Strassmann gives m. p. 148—149°). For reduction, 2·5 g. were refluxed with concentrated hydrochloric acid (10 ml.), alcohol (15 ml.), and tin (1·6 g.), more acid (5 ml.) and tin (2·6 g.) being added during 2 hours. After 3·5 hours the clear solution was decanted from unchanged tin, excess of alcohol was evaporated, the residues were made strongly alkaline, and the N-o-*xylylphthalimidine* was extracted in benzene. The crude material, decolorised in alcoholic solution and crystallised from aqueous alcohol, then formed colourless needles, m. p. 95·5—96·5° (1·7 g.) (Found: C, 81·0; H, 6·3; N, 5·9. $C_{16}H_{15}ON$ requires C, 81·0; H, 6·4; N, 5·9%).

(*b*) A mixture of (VI) (0·27 g.), acetic acid (5 ml.), and zinc (0·2 g.) was refluxed, and during 2 hours more acetic acid (3 ml.) and zinc dust (0·3 g.) were added. After a further hour the unchanged zinc was filtered from the hot liquid and washed with hot alcohol (2 × 5 ml.), and the filtrate diluted with water (50 ml.) and left until the product crystallised (0·18 g.; m. p. 93—95°). After crystallisation it had a m. p. identical with the product from (*a*) and a mixed m. p. showed no depression. The infra-red spectrum of a Nujol suspension showed a C=O vibration band at 1686 cm.$^{-1}$, but no OH band.

Oximinophthalimide from o-*Phthalaldehydic Acid*.—Oximinophthalimide has been prepared from phthalaldehyde and hydroxylamine (Beilstein's "Handbuch," 4th edn., vol. 21, p. 460). It can equally well be prepared from *o*-phthalaldehydic acid or 3-ethoxyphthalide. When either of these compounds (0·5 g.) and hydroxylamine hydrochloride (1·5 g.), pyridine (7·5 ml.), and ethanol (7·5 ml.) were heated together on a water-bath for 3 hours, oximinophthalimide was the main product. It (0·25 g.), m. p. 262—264° (decomp.), was isolated by evaporating most of the solvent, pouring into ice-cold dilute hydrochloric acid, and crystallising the colourless precipitate from methanol (Found: C, 59·5; H, 3·6; N, 17·2. Calc. for $C_8H_6O_2N_2$: C, 59·3; H, 3·7; N, 17·3%). A mixed m. p. with a specimen prepared from *o*-phthalaldehyde showed no depression. The following derivatives appear to be new: o-*phthalaldehyde bis*-2 : 4-*dinitrophenylhydrazone*, small, bright orange needles (from *o*-dichlorobenzene), m. p. 278—280° (decomp.) (Found: C, 48·5; H, 2·9; N, 22·7. $C_{20}H_{14}O_8N_8$ requires C, 48·6; H, 2·9; N, 22·7%), and *bis*-p-*aminoazobenzene* derivative (separated as a crystalline precipitate when concentrated ethanolic solutions of *o*-phthalaldehyde and *p*-aminoazobenzene were mixed and left for 3 hours at room temperature), orange-brown crystals (from *n*-butanol), m. p. 196—197·5° (decomp.) (Found: C, 78·2; H, 4·9; N, 17·0. $C_{32}H_{24}N_6$ requires C, 78·0; H, 4·9; N, 17·1%); the 2 : 4-*dinitrophenylhydrazone of* o-phthalaldehydic acid, small orange needles (from *n*-butanol), m. p. 264—266° (decomp.) (Found: C, 51·2; H, 3·2; N, 17·0. $C_{14}H_{10}O_6N_4$ requires C, 50·9; H, 3·1; N, 17·0%).

We are indebted to Dr. J. B. Willis for the measurements of the infra-red spectra and for their interpretation.

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41. *Infra-red Studies of the Salicylides and Cresotides.*

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The infra-red spectra of the salicylides and cresotides have been measured. The variations in the carbonyl-bond stretching frequency are used to obtain information about the configurations adopted by these compounds. The frequencies found support the structures proposed by Edgerley and Sutton on the basis of dipole-moment measurements. The carbonyl frequencies for a number of substituted phenyl esters are also presented and discussed.

As an elaboration of the chemical investigation of the salicylides and cresotides (Baker, Ollis, and Zealley, *J.*, 1951, 202; Baker, Gilbert, Ollis, and Zealley, *J.*, 1951, 210) and the measurement of their dipole moments (Edgerley and Sutton, *J.*, 1951, 1069), the infra-red spectra of the salicylides $\left[o\text{-}C_6H_4\langle^{O-}_{CO-}\right]_n$ and the corresponding cresotides derived from *o*-, *m*-, and *p*-cresotic acids (2-hydroxy-3-, 2-hydroxy-4-, and 2-hydroxy-5-methylbenzoic acids) have been measured.

Experimental

The spectra of the compounds listed in Tables 1, 2, and 3 were measured in the region of the carbonyl-bond stretching vibration (1700—1800 cm.$^{-1}$) with a Perkin–Elmer Model 12C spectrometer with a rock-salt prism. The substances were examined as suspensions in paraffin (Nujol), and as dilute solutions in carbon tetrachloride with a cell thickness of approx. 0·5 mm. Tetra-*p*-cresotide, hexasalicylide, and hexa-*m*-cresotide were not sufficiently soluble for their spectra to be measured in carbon tetrachloride solution.

Results and Discussion

The carbonyl-bond frequencies of the salicylides and cresotides are given in Table 1 (for solutions) and in Table 2 (for solids). In Table 3 are given the corresponding frequencies for several esters of related interest.

Table 1. *Carbonyl-bond stretching frequencies (in cm.$^{-1}$) for solutions in carbon tetrachloride (values in parentheses are for weak bands).*

	Di-	Tri-	Tetra-
Salicylides	1773	1749	1740
o-Cresotides	1777 (1769)	1764 (1748)	1736
m-Cresotides	1767	1739 (1749)	1736
p-Cresotides	1768	1742	—

Table 2. *Carbonyl-bond stretching frequencies (in cm.$^{-1}$) for solids.*

	Di-	Tri-	Tetra-	Hexa-
Salicylides	1751 (1764)	1730 (1743)	1730	1729
o-Cresotides	1751 (1762)	1746 (1729)	1729	—
m-Cresotides	1753 (1761)	1731	1730	1722
p-Cresotides	1753 (1768)	1724 (1746)	1725	—

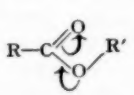
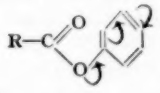
Table 3. *Carbonyl-bond stretching frequencies (in cm.$^{-1}$) for some esters in carbon tetrachloride solution.*

Ethyl acetate	1735	*p*-Dimethylaminophenyl acetate	1751
Phenyl acetate	1764	Phenyl benzoate	1738
p-Nitrophenyl acetate	1771		

Models show that compounds of the salicylide type can take up a number of interconvertible, strainless configurations. In order to obtain information from their infra-red spectra regarding the actual structure adopted it is necessary to consider bands which can be assigned to particular vibrations, variations in the frequency of which can be correlated

with steric effects. The only such bands are those produced by the carbonyl-bond stretching vibration with a frequency of 1720—1780 cm.$^{-1}$. The normal frequency for the carbonyl stretching vibration of an aliphatic ester in solution (*e.g.*, ethyl acetate, Table 3) is about 1735 cm.$^{-1}$ (Thompson and Torkington, *J.*, 1945, 640); this may be modified by the influence of the groups to which the ester group is attached. In an ester, R·CO_2R′, when R is a group which can conjugate with the carbonyl group, as in methyl benzoate, the frequency is reduced by about 10—20 cm.$^{-1}$ (Rasmussen and Brattain, *J. Amer. Chem. Soc.*, 1949, **71**, 1073). When R′ is unsaturated, as in phenyl acetate, the carbonyl frequency is raised to about 1765 cm.$^{-1}$ (Table 3). When both these effects are operative, as in phenyl benzoate, the carbonyl frequency might be expected to be somewhat above the normal value, and for phenyl benzoate it is found to be 1738 cm.$^{-1}$. This seems to be the normal value to be expected for the salicylide sand cresotides if the benzene rings exert their maximum effect.

The question arises why the carbonyl-bond stretching frequency for phenyl acetate should be so much higher than the normal value. In any ester R·CO_2R′ electromeric interaction may occur as in (I) provided that the atoms of the ester group ·CO_2R′ are coplanar. It has been suggested that if R′ is a phenyl group, this can interact with the ether-oxygen atom as in (II) and thus strengthen the carbonyl bond by opposing the effect shown below (I). However, as noted by Edgerley and Sutton (*loc. cit.*), interference

(I)   (II)

between the carbonyl-oxygen and the hydrogen atom in the *o*-position of the phenyl group prevents the coplanar configuration from being adopted, and the electromeric effect (II) should be very slight. The frequencies given in Table 3 for *p*-nitrophenyl acetate and *p*-dimethylaminophenyl acetate provide evidence that there is electromeric interaction between the ether-oxygen atom and its attached phenyl group. The differences produced by the *para*-substituents cannot be accounted for in terms of purely inductive effects but can be readily explained if a degree of interaction between the phenyl group and the ether-oxygen atom be assumed. The *p*-nitrophenyl group should then enhance the effect shown in (II) and so raise the carbonyl-bond frequency, while the *p*-dimethylaminophenyl group supports (I) and lowers the frequency. It seems likely, therefore, that the free pair of electrons of the ether-oxygen atom occupy an orbital which can have an appreciable component in a direction which allows interaction with the π-electrons of the benzene ring.

The carbonyl-bond stretching frequencies given by the dimers are found to be much higher than those of the trimers and tetramers. With the exception of tri-*o*-cresotide, the trimers and tetramers have their carbonyl-bond frequencies at about 1740 cm.$^{-1}$, while for the dimers it is at about 1770 cm.$^{-1}$ in carbon tetrachloride solution (see Table 1). Edgerley and Sutton (*loc. cit.*) have provided conclusive evidence that in the dimers the ester groups adopt the *cis*- rather than the more usual *trans*-form. It might be thought that the *cis*-configuration always leads to a high carbonyl frequency, but for the lactones which have been shown by Marsden and Sutton (*J.*, 1936, 1383) to adopt the *cis*-ester configuration, normal carbonyl frequencies are given if steric strain is absent (Rasmussen and Brattain, *J. Amer. Chem. Soc.*, 1949, **71**, 1073).

Since there is no strain in the *cis*-form of the dimers, normal bond lengths and intervalency angles being possible, an alternative explanation of the high frequencies of the carbonyl-bond vibration must be sought. The ester groups in the dimers differ from the ester groups in, for example, phenyl benzoate, in one other feature. Instead of the carbonyl bond being coplanar with the benzene ring to which it is directly attached, it is directed at an angle of almost 90° to it. Conjugation between the benzene ring and its adjacent carbonyl group is therefore considerably reduced. The observed carbonyl frequencies of the dimers should approach that of phenyl acetate (1766 cm.$^{-1}$), and there is very good agreement with this expected value.

The trimers give a carbonyl-bond frequency a little above the normal value, with the exception of tri-*o*-cresotide, for which the frequency is still higher. Dipole-moment measurements (Edgerley and Sutton, *loc. cit.*) show that the ester groups in these compounds have the normal *trans*-configuration and these moments may be interpreted in terms of the pyramid-type structure discussed previously (see Baker, Ollis, and Zealley, and Edgerley and Sutton, *locc. cit.*). Such a structure serves to reduce the strain and steric interference which would be present if the molecule had the completely planar configuration. In the pyramidal structure for trisalicylide and tri-*m*- and -*p*-cresotides, the carbonyl groups can still be coplanar with the benzene rings to which they are directly attached, so that the carbonyl-bond frequencies of trisalicylide and tri-*m*- and -*p*-cresotide would be expected to be very close to that of phenyl benzoate, and this is found to be so.

In tri-*o*-cresotide, the replacement of three hydrogen atoms by larger methyl groups would tend to cause the molecule to depart even more from the planar structure (*i.e.*, increase the height of the pyramid) so that excessive steric interference could be avoided. The observed increase in the carbonyl-bond frequencies of tri-*o*-cresotide may be explained either by a resultant decrease in resonance interaction between the benzene rings and the carbonyl groups or by increased strain.

The carbonyl frequencies of the tetramers indicate that the steric conditions are very similar to those present in phenyl benzoate, and that the bonds of the 16-membered ring are free from strain. A pyramidal configuration similar to that of the trimers would necessitate considerable departure from the normal intervalency angles and so should lead to the higher carbonyl-bond frequencies characteristic of strained ring systems. The infra-red results support the structure proposed by Baker, Ollis, and Zealley (*loc. cit.*) in which the four, almost planar, $O{\cdot}C_6H_4{\cdot}CO$ units are alternately above and below the plane of the four bridge-oxygen atoms. This type of structure for tetrasalicylide is compatible with its dipole moment.

Since most of the compounds when measured in solution give a single intense carbonyl band, it is very unlikely that they exist in solution as equilibrium mixtures of different forms, in spite of the fact that examination of models of these large-membered ring compounds shows that a large number of strainless configurations are possible. The presence of weak bands in association with strong carbonyl bands for *cis*-di- and tri-*o*-cresotide and for tri-*m*-cresotide makes the position less certain for these, but it may be explained in terms of overtones or combination bands.

The carbonyl frequencies given by the substances in the solid state are shown in Table 2. No conclusion can be drawn from the difference in carbonyl frequency for a particular solid and its solution, but the same inter-relations were shown by the solids as by their solutions. Splitting of the bands in the case of solids is frequently encountered; it may be due to differences of the environment of particular carbonyl groups within the crystal lattice, as well as to the factors noted above.

The author acknowledges the award of a scholarship by the Australian National University during the tenure of which this work was done. Thanks are due to Professor W. Baker, F.R.S., and Dr. W. D. Ollis who suggested the investigation and supplied the compounds, and to them, Dr. H. W. Thompson, F.R.S., Mr. L. Orgel, and Mr. G. Gilbert for valuable discussion.

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42. *The Oligosaccharides Synthesised from Maltose by* Escherichia coli.

By S. A. Barker and E. J. Bourne.

Washed cells of *E. coli* (Monod strain) have been incubated with maltose in the presence of iodoacetate, and the extra-cellular saccharides have been fractionated on a charcoal column. The fractions have been subjected to paper-chromatographic analysis, methylation and end-group assay, and oxidation with both hypoiodite and periodate. In addition, the molecular weights of their acetates have been determined. By these methods the saccharides have been shown to include glucose, maltose, and the lower members of the homologous series of glucose polymers containing the 1 : 4-α-glucosidic linkage.

In 1924, Grey (*Biochem. J.*, **18**, 712) reported that a strain of coliform bacteria, when incubated in a glucose medium, without aeration and in the presence of calcium carbonate to neutralise acids as produced, synthesised a starch-type polysaccharide, which could be extracted with aqueous oxalic acid. More recently, Monod and Torriani (*Compt. rend.*, 1948, **227**, 240) isolated from a strain of *Escherichia coli*, which was both maltose- and lactose-positive (Monod, Torriani, and Gribetz, *ibid.*, p. 315), an enzyme fraction which, under suitable conditions, catalysed the synthesis of starch-type products from maltose but not from glucose-1 phosphate or sucrose. It was clear that the enzyme system responsible for this synthesis did not include phosphorylase, the amylose-synthesising enzyme which is commonly found in plant and animal tissues and uses the Cori ester as its substrate. Neither was the active agent amylosucrase, an enzyme which has been shown by Hehre and his colleagues (*J. Bact.*, 1948, **55**, 197; *J. Biol. Chem.*, 1949, **177**, 267) to occur in *Neisseria perflava* and to catalyse the synthesis of a starch-type polysaccharide from sucrose. The French workers furnished evidence that the higher saccharides were in fact formed directly from maltose; the enzyme responsible was named by them "amylomaltase" and was shown to catalyse the reaction, n maltose $\rightleftharpoons$ n glucose + $(\text{glucose})_n$. Thus the new enzyme, like phosphorylase, amylosucrase, and other enzymes responsible for the synthesis of polysaccharides, was a transglycosidase. The average degree of polymerisation of the mixture of glucose polymers resulting from amylomaltase action is normally quite small, but it has been demonstrated by Monod and Torriani (*Ann. Inst. Pasteur*, 1950, **78**, 65) that products of sufficiently high molecular weight to give a blue stain with iodine are produced when the glucose formed in the synthesis is continuously removed by means of glucose oxidase. Another contribution towards the elucidation of this intriguing topic was made in 1949 by Doudoroff, Hassid, Putman, Potter, and Lederberg (*J. Biol. Chem.*, 1949, **179**, 921), who reported a study of the metabolism of maltose by a suspension of a mutant of *E. coli* (strain W-327), which, in contrast to Monod's strain, was incapable of utilising either lactose or glucose. They fractionated the saccharides formed by resting cells of the organism, in the presence of iodoacetate, on the basis of their relative solubilities in alcohol, after having first removed glucose and maltose by fermentation and inorganic materials by ion-exchange resins. The fractions thus obtained consisted of a series of dextrins, possessing 4—6 glucose units per molecule.

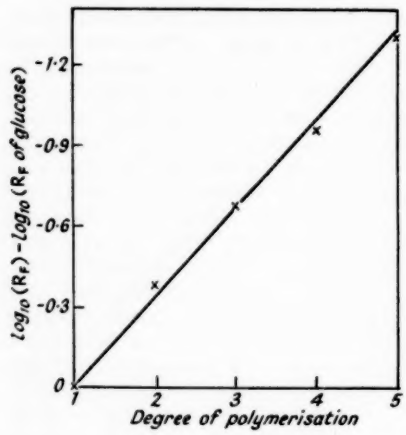
For some time we have been engaged, with our colleagues, in a study of Monod and Torriani's work. As part of that programme, we have examined the metabolism of maltose by *E. coli* (Monod strain) and have obtained results, reported herein, which are fully in accord with those obtained by Hassid and his co-workers, who used a different strain of the organism.

Washed cells of *E. coli* (Monod strain), which had been grown on a synthetic medium containing maltose as the sole source of carbon, were incubated with maltose in the presence of sodium hydrogen carbonate and sodium iodoacetate, under conditions similar to those employed by Hassid *et al.* (*loc. cit.*). After removal of the cells in the centrifuge, the supernatant liquid was fractionated by a purely chemical process, namely, by Whistler and Durso's method (*J. Amer. Chem. Soc.*, 1950, **72**, 677), later extended by Bailey, Whelan,

and Peat (*J.*, 1950, 3692), which involved passage of the solution through a charcoal column and subsequent elution of the column with aqueous solutions containing increasing concentrations of ethanol. In this way five fractions (A—E) were obtained (see Tables 1 and 4), the first as an aqueous solution and the rest as freeze-dried powders; they were not stained by iodine. In a second experiment, the fractions corresponding to C, D, and E were united and designated fraction F. As was expected, the amounts of inorganic material in the various eluates, as revealed by ash contents, decreased progressively with increasing concentration of ethanol (cf. Table 2).

Paper-chromatographic analysis of hydrolysates of the various fractions revealed, in each case, a single aldose component, having a R_F value identical with that of a reference glucose spot, and so it was very probable that each fraction was either glucose or an acid-labile derivative thereof. During paper-chromatographic analysis of the fractions themselves, five discrete spots, detectable by aniline hydrogen phthalate, were encountered; the distances moved by these spots (see Table 1), coupled with the corresponding figure for maltose (0·42), render it likely that the five spots represented glucose and the four lowest members of the homologous series of 1 : 4-α-linked glucose polymers, namely, maltose, maltotriose, maltotetraose, and maltopentaose. In conformity with this supposition, the plot of log (R_F) against degree of polymerisation was linear (see figure), as was the case also with a homologous series of dextrins obtained by Jeanes, Wise, and Dimler (*Analyt. Chem.*, 1951, **23**, 415) by α-amylolysis of amylose. Other slower-moving saccharides could not be isolated as discrete spots.

Paper chromatography of fractions A—E.



From the mode of its isolation, fraction A should have contained no sugars other than monosaccharides (cf. Whistler and Durso, *loc. cit.*); paper chromatography revealed glucose to be the only constituent, and optical-activity measurements indicated that this represented about 29% of the glucose units present initially in the maltose substrate. Fraction B should have consisted of disaccharide components and paper chromatography detected one such constituent, namely, maltose; this fraction had $[\alpha]_D$ (equil.) 100°, but, since its conversion into glucose by acid was only 76·5%, the true specific rotation of the carbohydrate moiety was 131°; the accepted value for maltose is 137°. Fraction C

TABLE 1. *Paper chromatography of the oligosaccharide fractions.*

Fraction	Ethanol (%) in aqueous ethanol used for elution	R_F value of sugar component / R_F value of glucose: Main components	Trace components
A	0	1·00	—
B	5	0·42	—
C	15	0·21	0·11, 0·42, 1·00
D	25	0·05, 0·11	1·00
E	35	0·00—0·05	1·00
F	15, 25, 35	0·05, 0·11, 0·21	—

contained one major constituent, probably maltotriose, together with traces of glucose, maltose, and maltotetraose; this fraction gave a 95·1% conversion into glucose and had $[\alpha]_D$ (equil.) 151°, which corresponded to 159° for the carbohydrate portion; values of $[\alpha]_D$ reported for maltotriose are 163° (Blom and Rosted, *Acta Chem. Scand.*, 1947, **1**, 233), 158° (French, Levine, Pazur, and Norberg, *J. Amer. Chem. Soc.*, 1950, **72**, 1746), 160° (Sugihara and Wolfrom, *J. Amer. Chem. Soc.*, 1949, **71**, 3357), and 167° (Bailey, Whelan,

and Peat, *loc. cit.*). Fraction D appeared to be a mixture of maltotetraose and maltopentaose, whilst fraction E contained mainly maltopentaose and higher dextrins; in each of these two fractions there was a trace of glucose.

Confirmatory evidence regarding the nature of the oligosaccharide fractions under examination was obtained by methylation and end-group assay, by oxidation with hypoiodite and periodate, and by measurement of the molecular weights of their acetates.

TABLE 2. *Purities of the oligosaccharide fractions.*

Fraction	% of glucose units in substrate appearing in product *		Ash (%)	$[\alpha]_D^{20}$ in water (equil.)	Converion (%) into glucose by acid
B	24·4		6·4	100°	76·5
C	16·9		2·6	151	95·1
D	14·1	34·5	1·5	171	93·6
E	3·5		3·1	169	91·2
F	36·6		0·9	170	91·1

* Based on the conversion (%) into glucose by acid.

Methylation and end-group assay was conducted on fraction F (equivalent to C + D + E), which was already believed to be essentially a mixture of malto-triose, -tetraose, and -pentaose. This was treated with sodium and methyl iodide in liquid ammonia at −70° (cf. Freudenberg and Boppel, *Ber.*, 1938, **71**, 2505) until the methoxyl content of the product attained a constant value (49·8%). Thereafter, hydrolysates of the methyl ether were analysed, qualitatively and quantitatively, by filter-paper chromatography. An aniline hydrogen phthalate spray revealed two aldose components, which had R_F values identical with those of 2 : 3 : 6-trimethyl and 2 : 3 : 4 : 6-tetramethyl glucose, used as standards on the same chromatogram. There was no trace of a dimethyl glucose constituent, thus proving that the oligosaccharides contained in fraction F were virtually unbranched. The average chain length, determined by a method similar to that described by Flood, Hirst, and Jones (*J.*, 1948, 1679), was about 3·7.

The molecular weights of fractions C, D, and E were determined by oxidation with hypoiodite under the rigid conditions specified by Hirst, Hough, and Jones (*J.*, 1949, 928). On the assumption that the oxidation was quantitative, the degrees of polymerisation were calculated to be 2·8, 4·0, and 5·1, respectively. There are strong grounds for the belief that this assumption was valid, first, because, in each case, there was a linear relation between the weight of oligosaccharide taken and the quantity of hypoiodite consumed (Table 5), and, secondly, because it is known that the oxidation proceeds quantitatively with glucose, methyl ethers of glucose (Hirst, Hough, and Jones, *loc. cit.*; Barker, Bourne, and Wilkinson, *J.*, 1950, 3027), maltose (Bailey, Whelan, and Peat, *loc. cit.*; Barker and Bourne, unpublished results), and maltotriose (French *et al.*, *loc. cit.*). Moreover, Levine, Foster, and Hixon (*J. Amer. Chem. Soc.*, 1942, **64**, 2331) found that the chain lengths of starch dextrins determined by oxidation with hypoiodite agreed with those calculated from the potassium contents of salts of the dextrinic acids produced in the oxidation. Hassid *et al.* (*loc. cit.*) recorded agreement between the chain length of their " fraction 2," determined by hypoiodite oxidation, and the molecular weight of its acetate, measured by a modification of Rast's method; a discrepancy between the two corresponding values for their " fraction 3 " was attributed by them to decomposition during the

TABLE 3. *Molecular sizes of the oligosaccharide fractions.*

Method of measurement	Degree of polymerisation			
	Fraction C	Fraction D	Fraction E	Fraction F
Methylation and end-group assay	—	—	—	3·7
Hypoiodite oxidation	2·8	4·0	5·1	—
Barger's method on acetate	2·8	4·1	—	—

cryoscopic measurements. We have ourselves obtained excellent agreement between the results of the hypoiodite oxidations of fractions C and D and the molecular weights of the acetates of these fractions, determined by Barger's method (see Table 3); there was insufficient of fraction E to permit the isolation of an acetate. Attempts to assess the chain lengths of the oligosaccharide fractions by determining the formic acid produced

during treatment with periodate (cf. Brown, Dunstan, Halsall, Hirst, and Jones, *Nature*, **1945**, **156**, 785) gave results some 25% higher than those of the methods already mentioned. This discrepancy is not regarded as significant because certain factors encountered during periodate oxidations of short-chain dextrins militate against accuracy; for example, it is difficult to choose the correct end-point for the final titration with alkali (cf. Meyer and Rathgeb, *Helv. Chim. Acta*, 1949, **32**, 1102), over-oxidation of reducing residues must be avoided (cf. Hassid and Potter, *J. Amer. Chem. Soc.*, 1948, **70**, 3488), and destruction of formic acid occurs if the pH falls too low (cf. Meyer and Rathgeb, *loc. cit.*).

The behaviour of fractions C, D, and E towards soya-bean β-amylase was in accordance with the foregoing conclusions (see Table 7), and with the observation of other workers that maltotriose is attacked only very slowly by this enzyme (cf. Bailey, Whelan, and Peat, *loc. cit.*; French *et al.*, *loc. cit.*). Fraction C, known to be essentially maltotriose, showed an increase in reducing power of only 3% in 17·5 hours and 14% in 41·5 hours. The corresponding figures for fraction D were 97 and 105%, and for fraction E 199 and 228%. Thus, in the last two cases there was an initial rapid reaction, followed by a relatively slow one; paper-chromatographic analysis revealed that, at the end of the first stage, the products were maltose and maltotriose, the latter being responsible for the subsequent slow reaction.

In conclusion, it has thus been shown that incubation of a washed culture of *E. coli* with maltose in the presence of iodoacetate afforded, in the extracellular solution, a mixture of glucose, unchanged maltose, and higher homologues of maltose. Of the glucose units present initially in the substrate approximately 29% were converted into free glucose, 24% remained as maltose, and 35% were converted into higher saccharides. An examination is now being made of a polysaccharide which is stored intracellularly by cultures of *E. coli* (Monod strain) when grown on a synthetic medium containing maltose; details of this work will be published later.

Experimental

Isolation of Escherichia coli.—*E. coli* (Monod strain) was grown at 35° for 22 hours in an aerated synthetic medium (6·5 l.), containing maltose as the sole carbon source, as described by Monod and Torriani (*loc. cit.*). The cells were collected in a Sharples continuous centrifuge and washed by the passage of distilled water (5 l.). The bacterial sludge was freeze-dried to a yellow powder (4·94 g.), which was stored at 1° until required.

Isolation of the Oligosaccharides synthesised from Maltose by E. coli.—A digest (40 c.c.), containing maltose hydrate (2·888 g.), freeze-dried *E. coli* (1·20 g.), sodium hydrogen carbonate (3·36 g.), and sodium iodoacetate (0·0166 g.), was incubated at 32° for 3 hours, during which time a stream of carbon dioxide was bubbled through the suspension. Excess of trichloroacetic acid was added, and the precipitate, collected by the centrifuge, was washed with 6% trichloroacetic acid solution (20 c.c.). The supernatant liquid and washings were combined and fractionated on a charcoal column (17 × 3·4 cm.) by Whistler and Durso's method (*loc. cit.*). The column was washed as shown in Table 4. Each of the eluates, with the exception of the first, was concentrated to a small volume (*ca.* 25 c.c.), filtered, and freeze-dried to a white solid, which was subsequently dried to constant weight at 60° in a vacuum over phosphoric oxide.

Table 4. *Yields of the oligosaccharide fractions.*

Eluant	Oligosaccharide product: Fraction	g.	Eluant	Oligosaccharide product: Fraction	g.
Water, 800 c.c.	A	—	25% Ethanol, 700 c.c. ...	D	0·402
5% Ethanol, 1400 c.c. ...	B	0·874	35% Ethanol, 400 c.c. ...	E	0·103
15% Ethanol, 900 c.c. ...	C	0·479			

In a duplicate experiment, in which maltose hydrate (2·908 g.) was employed, the fractions corresponding to fractions C, D, and E were united. The product (1·081 g.) was designated fraction F.

Examination of the Oligosaccharides.—(a) *Ash content.* The oligosaccharide (20 mg.) was heated in a micro-muffle until there was no further change in weight.

(b) *Optical activity.* The specific rotations were determined in aqueous solution (*c*, 0·5—1·0).

(c) *Paper-chromatographic analysis.* Spots of the de-ionised aqueous eluate, and of solutions (2%) of fractions B—F, together with reference spots of solutions (2%) of maltose and glucose,

were applied to a sheet of filter paper (Whatman No. 1), which was then irrigated for 66 hours with the upper phase of a mixture of *n*-butanol (40%), ethanol (10%), water (49%), and ammonia (1%). The sheet was dried and stained with aniline hydrogen phthalate, as described by Partridge (*Nature*, 1949, **164**, 443).

(d) *Reducing sugars liberated during acidic hydrolysis.* (i) Identification. The oligosaccharide (10 mg.) was heated at 100° for 5 hours with 2N-sulphuric acid (0·50 c.c.) in a sealed tube. The solution was diluted with water (2·00 c.c.) and freed from ions by means of an apparatus similar to that described by Consden, Gordon, and Martin (*Biochem. J.*, 1947, **41**, 590). The hydrolysate, together with a reference solution of glucose (2%), was submitted to paper-chromatographic analysis, as described above. The aniline hydrogen phthalate spray revealed only one component in each of the hydrolysates derived from fractions B—F; this component had an R_F value identical with that of the glucose reference spot. A second chromatogram, which had been irrigated for only 24 hours, failed to show any faster-moving component.

(ii) Determination of glucose. A sample (13—20 mg.) of the oligosaccharide was hydrolysed for 3 hours with 7% sulphuric acid at 100°, and the glucose produced was determined by cuprimetric titration, as described by Bourne, Donnison, Haworth, and Peat (*J.*, 1948, 1687). In the calculations of the percentage conversions into glucose, fractions B, C, D, and E were assumed to be di-, tri-, tetra-, and penta-saccharides, respectively. A small correction (1·5%) was made for the loss in reducing power which occurs when glucose itself is treated with acid under these conditions (Pirt and Whelan, *J. Sci. Food Agric.*, 1951, **2**, 224).

Methylation and End-group Assay of Fraction F.—(a) *Methylation.* Fraction F (0·942 g.) was treated with sodium and methyl iodide in liquid ammonia at −70°, according to the general method of Freudenberg and Boppel (*loc. cit.*). After four additions of the methylating reagents had been made, the ammonia was allowed to evaporate, with exclusion of moisture; the last traces of ammonia were removed at 55°/15 mm. Extraction of the residue with dry ether (Soxhlet) and evaporation of the extract afforded a glass (0·794 g.), having OMe 47·1%, and $[\alpha]_D^{17}$ +166° (*c*, 0·71 in chloroform). Part (0·721 g.) of this material was treated four more times with the methylating agents; the product, isolated as before, was again a glass (0·557 g.), having OMe 49·8%, and $[\alpha]_D^{17}$ +169° (*c*, 0·30 in chloroform). Further treatments with sodium and methyl iodide did not increase the methoxyl content.

(b) *Hydrolysis and end-group assay.* The hydrolysate was prepared and analysed by a method which was essentially that used by Flood, Hirst, and Jones (*loc. cit.*) and later by Hirst, Hough, and Jones (*loc. cit.*). Minor modifications introduced by Barker, Bourne, and Wilkinson (*loc. cit.*) were also incorporated in the method. Chromatography of the hydrolysate, as described above, showed two spots only; these had R_F values identical with those of 2 : 3 : 6-trimethyl and 2 : 3 : 4 : 6-tetramethyl glucose, used as standards on the same chromatogram. As a result of several independent quantitative determinations, based on oxidation with hypoiodite, it was calculated that the molecular composition of the mixture was trimethyl glucose 72·7 and tetramethyl glucose 27·3%; these values correspond to an average chain length of 3·7 glucose units for fraction F.

In control experiments, glucose and maltose hydrate were methylated with sodium and methyl iodide, and gave syrupy products, having OMe 60·9 and 53·7%, respectively. These products were subjected to methanolysis, hydrolysis, and paper-chromatographic analysis, as described above. The chromatogram obtained in the case of glucose showed an intense spot with the same R_F value as 2 : 3 : 4 : 6-tetramethyl glucose, and a very faint spot, which was probably due to a trimethyl glucose. The chromatogram derived from maltose showed two intense spots, corresponding in position to 2 : 3 : 6-trimethyl and 2 : 3 : 4 : 6-tetramethyl glucose, respectively.

Oxidation of Fractions C, D, *and* E *with Hypoiodite.*—A series of solutions of these fractions was oxidised with iodine in alkaline solution, and the excess of iodine was determined by sodium thiosulphate, as described by Hirst, Hough, and Jones (*loc. cit.*). The results quoted in Table 5 show that there was a linear relation in each case between the amount of oligosaccharide employed and the amount of iodine consumed. The chain lengths recorded in Table 5 were calculated on the assumption that the oxidation was stoicheiometric, and on the basis of the conversions into glucose shown in Table 2.

Acetylation of Fractions C *and* D.—In the standard method the saccharide (*ca.* 100 mg.) was suspended in a mixture of acetic anhydride (2·0 c.c.) and pyridine (2·0 c.c.) and left at room-temperature for 48 hours. Ice-water (50 c.c.) was added and, after 24 hours, the precipitate was collected by filtration. It was washed with water (100 c.c.) and dried at 60° in

TABLE 5. *Oxidation of fractions* C, D, *and* E *with hypoiodite.*

Weight (mg.)	Equiv. vol. (c.c.) of 0·01-N-$Na_2S_2O_3$	Chain length	Weight (mg.)	Equiv. vol. (c.c.) of 0·01N-$Na_2S_2O_3$	Chain length	Weight (mg.)	Equiv. vol. (c.c.) of 0·01N-$Na_2S_2O_3$	Chain length
Fraction C			*Fraction* D			*Fraction* E		
1·589	0·63	2·85	2·423	0·70	3·89	2·123	0·49	4·76
3·178	1·30	2·76	4·846	1·34	4·07	3·539	0·78	4·99
3·989	1·64	2·74	6·058	1·68	4·05	5·662	1·17	5·34
4·767	1·98	2·71	7·269	1·99	4·11	8·493	1·80	5·20
	Mean	2·77		Mean	4·03		Mean	5·07

a vacuum over phosphoric oxide. In order to avoid fractionation of the product, no attempt was made to crystallise it.

In a control experiment, maltose hydrate (110·1 mg.) gave a product (172 mg., 83%), $[\alpha]_D^{17}$ +57·3° (*c*, 0·70 in chloroform) (Found : Ac, 50·4. Calc. for $C_{28}H_{38}O_{19}$: Ac, 50·7%). β-Maltose octa-acetate has $[\alpha]_D^{20}$ +62·6° (in chloroform).

Acetylation of fraction C (98·5 mg.) gave a product (135 mg.), $[\alpha]_D^{17}$ +106·8° (*c*, 0·46 in chloroform) (Found : Ac, 49·4. Calc. for $C_{40}H_{54}O_{27}$: Ac, 49·0%).

In the same way, fraction D (99·4 mg.) afforded an acetate (157 mg.), $[\alpha]_D^{17}$ +127·7° (*c*, 0·71 in chloroform) (Found : Ac, 48·6. Calc. for $C_{52}H_{70}O_{35}$: Ac, 48·0%).

Molecular weights. The molecular weights of the acetylated oligosaccharides were determined by Barger's method (*J.*, 1904, 286), β-glucose penta-acetate being employed as the standard substance and ethyl acetate as the solvent. Alternate drops (5—7 mm. in length) of the standard solution and of a solution of the acetylated oligosaccharide were drawn into a capillary tube (length, 13 cm.; diameter, 1—2 mm.), and the extremities of the tube were sealed in a small flame. The lengths of the five centre drops were measured, with the aid of a travelling microscope, after 1 hour and again after 17 hours. For each acetate, the experiment was repeated several times, with glucose penta-acetate solutions of different concentrations. The isopiestic condition was determined by plotting the concentrations of the standard solutions against the algebraic difference of Δl (unknown) and Δl (known), where Δl was the summation of any increase or decrease in length occurring during the interval between the measure-

TABLE 6. *Molecular weights of the acetates of fractions* C *and* D.

Concn. of acetate (% by wt.)	Concn. of glucose penta-acetate (% by wt.)	Δl (unknown)	Δl (known)	Difference
Fraction C				
8·021	5·409	−0·836	+0·563	−1·399
8·021	4·222	−0·353	+0·086	−0·439
8·021	3·158	+0·030	−0·230	+0·260
8·021	2·646	+0·545	−0·329	+0·874
Fraction D				
10·56	5·409	−0·955	+0·581	−1·536
10·56	4·222	−0·237	+0·128	−0·365
10·56	3·776	−0·159	+0·101	−0·260
10·56	3·158	+0·143	−0·071	+0·214
10·56	2·646	+0·266	−0·227	+0·493

ments. The results are recorded in Table 6. They correspond to molecular weight, C 920 and D 1270, and degree of polymerisation, C 2·8 and D 4·1.

β-*Amylolysis of the Oligosaccharides.*—(a) *Determination of reducing power.* The digests employed contained the oligosaccharide (*ca.* 8 mg. in 2 c.c. of water), M-acetate buffer (pH 4·7; 1 c.c.), and a 0·3% solution (1 c.c.) of soya-bean β-amylase (prepared by the method of Bourne, Macey, and Peat, *J.*, 1945, 882). The reducing powers of the digests were determined at intervals by means of the Shaffer–Hartmann copper reagent (*J. Biol. Chem.*, 1921, **45**, 365), a correction being applied in each case for the reducing power of the enzyme sample. The results are shown in Table 7.

(b) *Chromatographic analysis of* β-*amylolysis products.* After the above digests had been incubated for 17·5 hours, aliquot portions were subjected to paper-chromatographic analysis,

TABLE 7. *Changes in reducing power* (*as % of that of maltose*) *during* β-*amylolysis.*

Time (hours) :	0	17·5	41·5
Fraction C	61·9	63·7	70·3
Fraction D	40·9	80·7	83·7
Fraction E	25·8	77·2	84·4

as described above. In each case, aniline hydrogen phthalate spray revealed two components; these had R_F values identical with those of maltose and maltotriose, used as standards on the same chromatogram. The proportion of maltotriose, judged from the intensities of the stains, appeared to be greatest in the products from fraction C and least in those from fraction E.

The authors are indebted to Professor M. Stacey, F.R.S., for his interest and advice during the course of this work, to Dr. J. Monod of the Pasteur Institute for kindly supplying the culture of *E. coli* used, and to the Royal Society for the award of a scholarship to one of them (S. A. B.). The expenses of the investigation were met by a grant from the Nuffield Foundation.

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43. *Diamidides. Part II. 2 : 4-Diaryltriazapentadienes.**

By D. A. PEAK.

A general method for the preparation of *N*-thiobenzoylbenzamidines (IV) has been developed and they have been shown to undergo fission by ammonia or primary amines, mainly by reaction at the amidino-carbon atom. When benzamidines are used instead of amines, the reaction gives rise to 2 : 4-diaryltriazapentadienes (III),* isolated as their relatively stable hydrated hydrochlorides.

N-Benzoylbenzamidine also undergoes fission but considerably more slowly than its thio-analogue. The rate of reaction is much increased by the phenyl group on *N'*-benzoyl-*N*-phenylbenzamidine, although side-reactions occur. *N*-Benzoyl-*N*-phenylbenzamidine also reacts rapidly but probably by a different mechanism. *N*-2 : 4-Dinitrobenzoylbenzamidine reacts abnormally while *N*-benzenesulphonylbenzamidine is quite stable to ammonia. The mechanisms of these reactions are discussed.

Very small yields of 2 : 4-diphenyl-1 : 3 : 5-triazapenta-1 : 3-diene (III*a*) have been obtained by the reaction of benzamidine with ethyl benzimidate at 100° and by the pyrolysis of benzamidine in boiling benzene.

IN Part I of this series * the preparation and properties were described of a number of diamidides of the types (I) and (II), in which at least one of the R, R', or R'' groups was Ph, and it was concluded that the conjugated structure of the latter type is an important

(I) NR:CPh·NR'·CPh:NR'' NRR'·CPh:N·CPh·NR'' (II)

factor in stabilising the molecule or its ion. It therefore appeared probable that conjugated diamidides of the type (III), in which the nitrogen atoms carry no aryl or alkyl substituents, should be capable of existence. The present paper describes the preparation of a number of such diamidides, isolated as their fairly stable hydrated hydrochlorides.

NH_2·CAr:N·CAr':NH	Ph·CS·N:CAr·NH_2
(III*a*; Ar = Ar' = Ph)	(IV*a*; Ar = Ph)
(III*b*; Ar = Ar' = C_6H_4·OMe-*p*)	(IV*b*; Ar = C_6H_4·OMe-*p*)
(III*c*; Ar = Ar' = C_6H_4Cl-*p*)	(IV*c*; Ar = C_6H_4Cl-*p*)
(III*d*; Ar = Ph, Ar' = C_6H_4·OMe-*p*)	(IV*d*; Ar = C_6H_4·SO_2Me-*p*)

The methods of Part I (*loc. cit.*) were not adaptable to the synthesis of the diamidides (III) owing to the instability of benzimidoyl chlorides or benzenesulphonates containing an unsubstituted imido-group. Of alternative routes, a promising one appeared to be the desulphurising amination of *N*-thiobenzoylbenzamidines (IV). The preparation of *N*-thiobenzoylbenzamidine (IV*a*) by condensation of thiobenzamide with phenyl cyanide in saturated ethereal hydrogen chloride was first described by Matsui (*Zentr.*, 1911, I, 982). He ascribed to it the structure of a dibenzimidoyl sulphide (NH:CPh·S·CPh:NH), but Rivier and Schneider (*Helv. Chim. Acta*, 1920, **3**, 115) preferred the structure (IV) on

* For nomenclature see Part I, Cooper, Partridge, and Short, *J.*, 1951, 391.

physical evidence and Ishikawa (*J. Chem. Soc. Japan*, 1921, **42**, 579; *Abstr.*, 1921, **120**, 728) later provided chemical evidence for this structure. In subsequent papers (*Abstr.*, 1923, **124**, 926; *et seq.*) he extended the reaction to other thioamides and cyanides.

The preparation of *N*-thiobenzoylbenzamidine (IV*a*) itself was found to proceed smoothly by this method. The crude hydrochloride obtained from the condensation invariably contained thiobenzamide (presumably as its hydrochloride) which proved a troublesome impurity in the final product. Methods were therefore developed for the removal of the thiobenzamide by taking advantage of the greater basicity of the *N*-thiobenzoylbenzamidine. Separation by differential solubility in alkali was impracticable owing to the rapid destruction of the compound under these conditions.

When, however, the condensation was attempted with substituted thiobenzamides and phenyl cyanides, *e.g.*, *p*-methoxythiobenzamide and *p*-methoxyphenyl cyanide, it failed owing to the almost complete precipitation of the thioamide as its hydrochloride. Attempts to avoid this in model experiments with thiobenzamide and phenyl cyanide were unsuccessful. The ether could not be replaced by other compatible solvents, such as dioxan or chloroform, and the condensation also failed in anhydrous liquid hydrogen fluoride.

A more general method for the preparation of *N*-thiobenzoylbenzamidines was found in the following route, starting from *N*-benzoylbenzamidines which are readily available by the method of Titherley and Hughes (*J.*, 1911, **99**, 1507):

$$\underset{\text{(V)}}{\text{Ph·CO·NH·CAr:NH,HCl}} \xrightarrow{PCl_5} \underset{\text{(VI)}}{\text{Cl·CPh:N·CAr:NH}} \xrightarrow[H_2S]{NEt_3} \text{(IV)}$$

The *N*-benzoylbenzamidine was converted into its hydrochloride (V), which with phosphorus pentachloride in chloroform yielded the imidoyl chloride (VI). The latter was then converted without purification into the *N*-thiobenzoylbenzamidine by a chloroform solution of anhydrous triethylamine saturated with dry hydrogen sulphide. A somewhat similar method has been used by Kendall (B.P. 408,638) for the preparation of *N*-arylthioacylamides. This method afforded *N*-thiobenzoylbenzamidine (IV*a*), *p*-methoxy-*N*-thiobenzoylbenzamidine (IV*b*) and *p*-chloro-*N*-thiobenzoylbenzamidine (IV*c*). In the case of *p*-methylsulphonyl-*N*-thiobenzoylbenzamidine (IV*d*), although the characteristic red colour of the reaction mixture indicated its formation, the compound could not be isolated because of its rapid fission under the conditions of the experiment to *p*-methylsulphonylthiobenzamide.

With aniline and mercuric oxide, *N*-thiobenzoylbenzamidine (IV*a*) readily yielded 1 : 2 : 4-triphenyl-1 : 3 : 5-triazapenta-1 : 3-diene (II; R = R′ = H, R″ = Ph). When, under the same conditions, alcoholic ammonia was used with mercuric oxide, mercuriammonium chloride, or lead hydroxide as desulphurising agent, the only products isolated were benzamidine (as picrate), thiobenzamide, cyaphenin (triphenyltriazine), an unidentified base $C_{20}H_{20}N_4S$ (as picrate), and 3 : 5-diphenyl-1 : 2 : 4-thiadiazole. The last product is a known oxidation product of thiobenzamide (Hofmann, *Ber.*, 1869, **2**, 646) and of *N*-thiobenzoylbenzamidine (Matsui, *loc. cit.*). The reason for the failure in this case was soon apparent in the rapid fission of the *N*-thiobenzoylbenzamidine with alcoholic ammonia in the absence of mercuric oxide, the red colour of the solution being discharged in a few minutes with the production of thiobenzamide and benzamidine. Under the same conditions *N*-thiobenzoylbenzamidine is stable to aniline for several days.

Thiobenzamide and benzamidine could arise by the fission of the molecule at either bond (*a*) or bond (*b*) or simultaneously at both:

$$\begin{array}{lll} NH_2\text{—}H & H\text{—}NH_2 \\ \text{Ph·CS—}NH & NH\text{—CPh:NH} \\ \text{Ph·CH}_2\text{·NH—}H & H\text{—NH·CH}_2\text{Ph} \end{array}$$

(*a*) (*b*)

When the fission was carried out with benzylamine (1 mol.) the reaction proceeded rapidly at room temperature and the products isolated were thiobenzamide (58·5%), *N*-benzylbenzamidine (78% as picrate), *N*-benzylthiobenzamide (3·5%), and benzamidine (1·2% as picrate). Under the mild conditions of the experiment it is unlikely that any substantial amount of either *N*-benzylbenzamidine or *N*-benzylthiobenzamide could have arisen as secondary product by the reaction of benzylamine with benzamidine and thiobenzamide respectively. Fission must therefore occur predominantly at (*b*).

It therefore appeared probable that fission with benzamidine instead of benzylamine would afford the required diamidide. This proved to be the case. Benzamidine (1 mol.) gradually decolourised a solution of *N*-thiobenzoylbenzamidine in ether with the formation of thiobenzamide and 2 : 4-diphenyl-1 : 3 : 5-triazapenta-1 : 3-diene (III*a*) which could be isolated from the reaction mixture either as its hydrochloride hemihydrate or as its picrate. The hydrochloride was a colourless solid of low solubility in water (*ca.* 0·4%) and of sufficient stability to be crystallised from water or ethanol without serious loss. In neutral aqueous solution it gradually deposited cyaphenin. In dilute hydrochloric acid it was rapidly converted into dibenzoylamine, a confirmation of its structure. In ethanol, the band at 247 mμ ($\varepsilon = 1{\cdot}73 \times 10^4$) in its ultra-violet absorption spectrum disappeared after several days, being replaced by a stronger band at 228 mμ ($\varepsilon = 2{\cdot}31 \times 10^4$). The curve had only a slight inflexion at *ca.* 280 mμ and the band at 330—345 mμ, associated in Part I (*loc. cit.*) with conjugation, was entirely absent. It therefore appears that both colour and this band are to be associated with the presence of at least one anilo-group in the conjugated system, since, in view of its comparative stability, the diamidide (III*a*) presumably exists in the conjugated form.

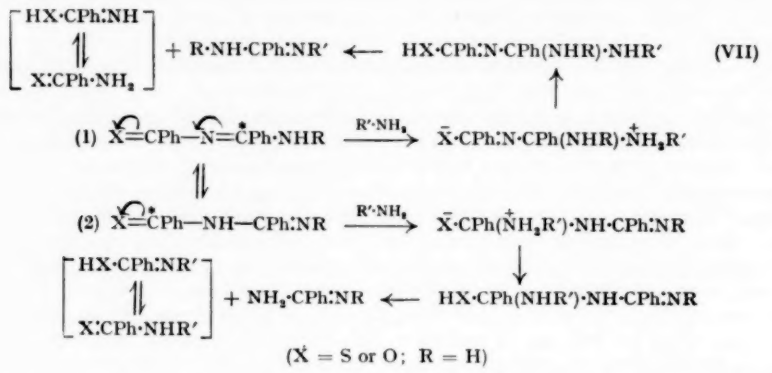
The hydrochloride melted partly at 204—206° to a turbid liquid which cleared sharply at 230°. These phenomena are due to its decomposition at its melting point into benzamidinium chloride, phenyl cyanide, and cyaphenin, the clearing at 230° presumably being due to the melting of the cyaphenin. The picrate, similarly, partly melted at 188° to a turbid mixture of benzamidinium picrate and cyaphenin which cleared sharply at 230°. These decompositions are entirely analogous to the decomposition of 1 : 2 : 4-triphenyl-1 : 3 : 5-triazapenta-1 : 3-diene (II; R = R′ = H, R″ = Ph) to phenyl cyanide and *N*-phenylbenzamidine (Part I, *loc. cit.*). The free diamidide (III*a*) was isolated only as an oil which gradually deposited crystals of cyaphenin.

A similar procedure gave 2 : 4-di-*p*-methoxyphenyl-1 : 3 : 5-triazapenta-1 : 3-diene (III*b*) and 2 : 4-di-*p*-chlorophenyl-1 : 3 : 5-triazapenta-1 : 3-diene (III*c*) hydrochlorides as their hydrate and hemihydrate respectively. Reaction of *p*-methoxy-*N*-thiobenzoylbenzamidine (IV*b*) with benzamidine gave the mixed diamidide, 2-*p*-methoxyphenyl-4-phenyl-1 : 3 : 5-triazapenta-1 : 3-diene (III*d*), as its hydrochloride hydrate.

Neither *N*-phenyl- nor *N*-benzyl-benzamidine reacted with *N*-thiobenzoylbenzamidine at room temperature. In boiling benzene *N*-phenylbenzamidine reacted slowly with the formation of cyaphenin and a small yield of 1 : 2 : 4 : 5-tetraphenyl-1 : 3 : 5-triazapenta-1 : 3-diene (II; R = H, R′ = R″ = Ph) instead of the expected 1 : 2 : 4-triphenyl-1 : 3 : 5-triazapenta-1 : 3-diene (II; R = R′ = H, R″ = Ph). This could be accounted for in two ways. The triphenyldiamidide, as a mixed anhydride in the ammono-system, could disproportionate to a mixture of the tetraphenyldiamidide and the diphenyldiamidide (III*a*), the latter being unstable under the conditions of the experiment. Alternatively, also in its capacity as a mixed anhydride in the ammono-system, the triphenyldiamidide could " acylate " unchanged *N*-phenylbenzamidine to the tetraphenyldiamidide with the liberation of benzamidine.

N-Benzoylbenzamidine underwent the same type of reactions as its thio-analogue but at a much slower rate. Over half was recovered unchanged after 5 days' reaction with alcoholic ammonia. After 7 days' reaction with benzylamine (1 mol.), *N*-benzylbenzamidine (40·5% as picrate) was isolated as the major fission product. The complementary product, benzamide, was isolated although in poor yield. The alternative fission products, *N*-benzylbenzamide and benzamidine (as picrate), were also isolated in minor quantities. Fission thus occurs preponderatingly at the amidino-carbon–nitrogen bond as in the case of the thio-compound. The following sequences of reactions probably

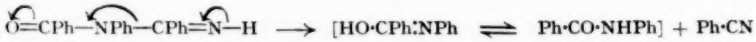
occur in both cases, (1) being predominant because of the greater proportion of the conjugated tautomer:



The greater reactivity of the thio-compound may be ascribed to the greater electromeric effect of the sulphur atom than of the oxygen atom.

It seemed possible that reactivity of the *N*-benzoylbenzamidines might be increased by the introduction of substituents which would promote electron deficiency at the carbon atoms marked above with an asterisk. In agreement with this, *N'*-benzoyl-*N*-phenylbenzamidine (X = O, R = Ph above), in which the *N*-phenyl group might be expected to produce this effect by reducing the electron-donor capacity of the nitrogen atom, reacted more rapidly than *N*-benzoylbenzamidine with ammonia. *N*-Phenylbenzamidine (30% as picrate) and benzamide (19%) were isolated as fission products. In this case, however, part of the reaction takes an alternative course and one of the products of reaction was *N*-benzoylbenzamidine (32% as picrate). This could be formed by the loss of aniline instead of benzamide from the hypothetical intermediate (VII; X = O, R = Ph, R' = H). In a similar manner, reaction with benzylamine gave *N*-phenylbenzamidine (17%) and *N'*-benzoyl-*N*-benzylbenzamidine (55·5%).

N-Benzoyl-*N*-phenylbenzamidine also reacted rapidly with alcoholic ammonia, the major product being benzanilide (68%). The reaction can scarcely be one of simple fission since the complementary fission product, benzamidine, could be isolated in only 2% yield (as picrate). Since phenyl cyanide was also identified qualitatively as a reaction product, this suggests the direct decomposition of *N*-benzoyl-*N*-phenylbenzamidine to benzanilide and phenyl cyanide in a manner exactly analogous to that postulated in Part I for the decomposition of the unstable 1 : 2 : 3 : 4-tetraphenyl-1 : 3 : 5-diazapenta-1 : 4-diene (I; R = R' = Ph, R'' = H):



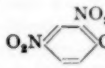
The isolation of *N*-benzoylbenzamidine (2% as picrate) indicates a certain amount of isomerisation of *N*-benzoyl-*N*-phenyl- to *N'*-benzoyl-*N*-phenyl-benzamidine during the reaction. The *N*-phenylbenzamidine isolated (5% as picrate) could arise from either isomer.

N'-Benzoyl-*N*-phenylbenzamidine used in the above experiments was prepared by the method of Wheeler, Johnson, and McFarland (*J. Amer. Chem. Soc.*, 1903, **25**, 787). Its preparation has also been reported by Beckmann and Sandel (*Annalen*, 1897, **296**, 293) by the action of aniline on *N*-benzoylbenzamidine at the boiling point of aniline. In view of the reactions of *N*-benzoylbenzamidine recorded above, this seemed an unexpected result. Repetition of this work proved it to be incorrect. No *N'*-benzoyl-*N*-phenylbenzamidine could be isolated and the products were the expected ones, *viz.*, benzamide (72%), benzanilide (3%), *N*-phenylbenzamidine (7·5%), *NN'*-diphenylbenz-

amidine (5·5%), and cyaphenin. The unexpectedly low yield of *N*-phenylbenzamidine and the considerable evolution of ammonia during the reaction indicate that the reaction is more complex than one of simple fission. The *NN'*-diphenylbenzamidine presumably arose as a secondary product from the reaction of *N*-phenylbenzamidine and aniline or by fission of the initially formed *N'*-benzoyl-*N*-phenylbenzamidine by aniline.

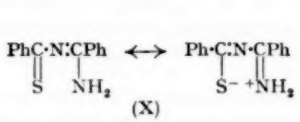
Wheeler *et al.* (*loc. cit.*) showed that the primary product of benzoylation of *N*-phenylbenzamidine was *N*-benzoyl-*N*-phenylbenzamidine. So readily does this rearrange to *N'*-benzoyl-*N*-phenylbenzamidine, particularly under the catalytic influence of traces of acid, that great difficulty was experienced in the preparation of a pure sample by their method. It was found that benzoylation in chloroform in the presence of an excess of triethylamine readily afforded a pure product.

N-Phenylbenzamidine in ether with benzoyl chloride (1 mol.) gave *NN'*-dibenzoyl-*N*-phenylbenzamidine (VIII), the structure of which was shown by its hydrolysis to a mixture of benzanilide and dibenzoylamine. It reacted fairly rapidly with alcoholic ammonia to give benzanilide and *N*-benzoylbenzamidine.

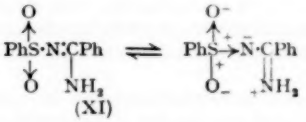
(VIII) $Ph{\cdot}CO{\cdot}N{:}CPh{\cdot}NPh{\cdot}COPh$  (IX)

In a further attempt to increase the reactivity of the molecule by the introduction of an electrophilic group, *N*-2 : 4-dinitrobenzoylbenzamidine (IX) was prepared. It possessed unusual properties in that, although nearly colourless in the crystalline form, it melted to a deep red liquid, becoming colourless again on solidifying. Rapid evaporation of its pale yellow solution in ether also produced red films which quickly became colourless. Completely satisfactory analyses were not obtained. With alcoholic ammonia, purple tars were produced from which no recognisable fission products could be isolated.

N-Benzenesulphonylbenzamidine, in spite of the strong electrophilic properties of the benzenesulphonyl group, did not react with alcoholic ammonia, no benzamidine or benzenesulphonamide being detectable after 2 days. This result, although at first sight unexpected, is in fact in accord with theoretical predictions. The electron drifts postulated above for the first stage of fission of *N*-benzoylbenzamidines would, if carried to completion, result in the canonical structure (X) for *N*-thiobenzoylbenzamidine. The corresponding structure (XI) for *N*-benzenesulphonylbenzamidine involves a negative charge on the central nitrogen atom which would tend to reduce the electron deficiency of the adjacent carbon atom.



(X)



(XI)

Before the work described above, an unsuccessful attempt had been made to prepare 2 : 4-diphenyl-1 : 3 : 5-triazapenta-1 : 3-diene (III*a*) by the reaction of ethyl benzimidate with benzamidine on analogy with the formation of amidines from imidic esters and ammonia :

$$NH{:}CPh{\cdot}OEt + NH_2{\cdot}CPh{:}NH \longrightarrow NH_2{\cdot}CPh{:}N{\cdot}CPh{:}NH + EtOH$$

With the recognition of the low solubility of the hydrochloride of the diamidide (III*a*) and its extremely characteristic melting-point behaviour which would permit easy isolation and identification of small quantities of the compound, this reaction was reinvestigated. It was found that no appreciable reaction occurred in ether after several days either at room temperature or at the boiling point. Heating the reactants at 100° in the absence of solvent, however, gave, besides cyaphenin, a small amount (2·6%) of the diamidide (III*a*) hydrochloride. It appears certain that under these conditions a considerable proportion of the diamidide would be destroyed. To cover the possibility of an unfavourable equilibrium, the experiment was repeated in refluxing ether, the condensate being returned to the reaction mixture over sodium wire to remove ethanol continuously.

No diamidide formation was detected. A similar procedure in benzene gave a small amount (1·05%). In neither experiment was the sodium appreciably attacked. Finally, it was found possible to obtain a small amount of the diamidide (III*a*) hydrochloride (0·35%) simply by heating benzamidine in benzene solution under reflux. It is presumably formed by elimination of a molecule of ammonia from two molecules of benzamidine. The significantly lower yield in this case makes it appear probable that ethyl benzimidate, when present, plays some part in the formation of the diamidide.

Experimental

*N-Benzoyl-*p*-methoxybenzamidine.*—*p*-Methoxybenzamidinium chloride (28 g.) was shaken with water (28 c.c.), 5N-sodium hydroxide (56 c.c.), and chloroform (80 c.c.) until all solid had disappeared. The chloroform layer was separated and the aqueous layer re-extracted with further chloroform (3 × 80 c.c.). The combined chloroform extracts were dried (Na_2SO_4), treated with charcoal, and filtered. An aliquot was estimated by titration with 0·1N-hydrochloric acid, and the residue (290 c.c. containing 19·13 g. of *p*-methoxybenzamidine) was mixed with phenyl benzoate (25·25 g., 1 mol.). Crystalline solid separated after 30 minutes but redissolved overnight. The chloroform was then removed *in vacuo* at <50° and the residue heated at 50° for 4 hours. The mixture was cooled and dissolved in ether (300 c.c.), and the solution was extracted with ice-cold 0·67N-hydrochloric acid (900 c.c.). Basification of the aqueous acid layer at 0° with 5N-sodium hydroxide precipitated an oil which quickly solidified. Crystallisation of the crude solid (26 g.; m. p. 101—103°) from ethanol afforded *N-benzoyl-*p*-methoxybenzamidine* (19·2 g.) as square plates, m. p. 104—105° (Found: N, 10·8. $C_{15}H_{14}O_2N_2$ requires N, 11·0%).

*N-Benzoyl-*p*-chlorobenzamidine.*—*p*-Chlorobenzamidinium benzenesulphonate (3·13 g.) (Oxley, Partridge, Robson, and Short, *J.*, 1946, 763) was dissolved in hot water (75 c.c.), the solution was cooled, and 5N-sodium hydroxide (3 c.c.) added. The precipitated amidine was taken up in chloroform (3 × 50 c.c.), estimated by titration of an aliquot, and condensed as before with phenyl benzoate (1·1 mols.), finally at 70° for 3 hours. Crystallisation of the crude base (1·88 g.) from light petroleum (b. p. 80—100°) gave *N-benzoyl-*p*-chlorobenzamidine* (1·40 g.) as small prisms, m. p. 121—122° (Found, after drying at 100°: N, 10·8. $C_{14}H_{11}ON_2Cl$ requires N, 10·8%).

*N-Benzoyl-*p*-methylsulphonylbenzamidine.*—*p*-Methylsulphonylbenzamidine (10·7 g.) and phenyl benzoate (10·0 g.) were ground together and the mixture was heated at 70—75° for 6 hours. Gradual melting and resolidification occurred during this period, and a strong smell of phenol developed. The product was dissolved in hot ethanol (110 c.c.). On cooling, *N-benzoyl-*p*-methylsulphonylbenzamidine* separated as prisms (11·2 g.), m. p. 161—162° raised to 163—164° by recrystallisation from ethanol (Found: N, 9·0. $C_{15}H_{14}O_3N_2S$ requires N, 9·3%). Concentration of the ethanolic mother-liquor gave a second *substance*, probably *NN'*-dibenzoyl-*p*-methylsulphonylbenzamidine, m. p. 223—224° (Found: N, 7·0. $C_{22}H_{18}O_4N_2S$ requires N, 6·9%).

Phenyl 2:4-Dinitrobenzoate.—A mixture of 2:4-dinitrobenzoic acid (27·2 g.), phenol (12·0 g.), and phosphorus oxychloride (27·2 g.) was heated slowly under reflux. Evolution of hydrogen chloride did not begin until 115° and was complete in 40 minutes. The product was poured into water, the water decanted, and the semi-solid residue dissolved in ether. The ethereal solution was washed twice with water and then with an excess of dilute sodium hydrogen carbonate solution. It was dried and concentrated to *ca.* 75 c.c. *Phenyl 2:4-dinitrobenzoate* (15·7 g.) slowly crystallised in prisms, m. p. 82—83° unchanged by further crystallisation from ether (Found: N, 9·35. $C_{13}H_8O_6N_2$ requires N, 9·7%).

N-(2:4-Dinitrobenzoyl)benzamidine.—Phenyl 2:4-dinitrobenzoate (11·50 g.) was added to a solution of benzamidine (4·8 g., 1 mol.) in pure chloroform (160 c.c.). A deep purple colour immediately developed. The condensation was completed as usual by heating the mixture at 60° for 3 hours in the absence of chloroform. The deep purple product was dissolved in acetone (50 c.c.) and acidified with 5N-ethanolic hydrogen chloride which precipitated a pale brown crystalline solid (11·4 g.). Recrystallisation of a small portion of this from methanol–ether afforded *N-(2:4-dinitrobenzoyl)benzamidinium chloride* as almost colourless prisms, m. p. 194—196° (Found: N, 16·5. $C_{14}H_{11}O_5N_4Cl$ requires N, 16·0%). The remaining crude chloride was shaken with chloroform and dilute sodium hydrogen carbonate. Evaporation of the chloroform left a deep purple liquid which dissolved in ether to give a pale yellow solution. Concentration of the ether solution gave the free *base* as pale-yellow prisms, m. p.

108—110° (Found : N, 17·25, 17·1. $C_{14}H_{10}O_5N_4$ requires N, 17·8%). The compound melted to a deep red liquid and rapid evaporation of solutions produced red oils which became pale yellow on storage.

Preparation of N-*Thiobenzoylbenzamidine from Thiobenzamide and Phenyl Cyanide*.—A solution of thiobenzamide (10·28 g.) and phenyl cyanide (7·73 g.) in dry ether (350 c.c.) was saturated at 0° with dry hydrogen chloride and kept at room temperature. A red oil rapidly separated which slowly crystallised. After 5 days the orange-yellow crystals of *N*-thiobenzoylbenzamidinium chloride were separated and washed by decantation. The crude product (19·1 g.), contaminated with thiobenzamide, probably in the form of its hydrochloride, was triturated with 2N-hydrochloric acid (50 c.c.) to convert the latter into free thiobenzamide, filtered, and washed on the filter with acetone. The residual solid (17·0 g.) was shaken with ether and an aqueous solution of potassium hydrogen carbonate (5 g.) until all the solid was in solution. The deep red ethereal layer was dried (Na_2SO_4) and evaporated *in vacuo*. Recrystallisation of the residue from benzene–light petroleum (b. p. 80—100°) gave the pure compound as long red needles (10·72 g., 59·5%).

Alternatively, the thiobenzamide could be removed by extracting the crude free bases into ether as above and reprecititating the *N*-thiobenzoylbenzamidinium chloride by the addition of ethanolic hydrogen chloride. Under these conditions, the thiobenzamide was not reprecipitated. The yield of pure material was the same by both methods.

Preparation of N-*Thiobenzoylbenzamidines from* N-*Benzoylbenzamidines*.—*N*-Benzoylbenzamidine (2·0 g.) (Titherley and Hughes, *loc. cit.*) was dissolved in acetone (10 c.c.), and ethanolic hydrogen chloride was added until the solution was acid to moist Congo-red paper. Addition of ether to incipient turbidity caused the separation of the hydrochloride in small needles (2·2 g.). This was suspended in pure chloroform (10 c.c.) and heated under reflux with phosphorus pentachloride (2·4 g.). Hydrogen chloride was evolved and a clear yellow solution resulted after 15 minutes. The solution was evaporated *in vacuo* and traces of phosphorus halides were removed by evaporating twice after the addition of dry toluene. The residual yellow oil was redissolved in pure chloroform (10 c.c.) and added drop-wise at 0° to a solution of triethylamine (1·7 g., 2 mols.) in pure chloroform (20 c.c.) which had previously been saturated with dry hydrogen sulphide at 0°. An immediate red colour developed. Hydrogen sulphide was passed through the solution during the addition and subsequently for a further 3 hours. The clear red solution was kept overnight at room temperature and then washed with water. The chloroform was separated from the aqueous layer and a small interfacial layer of dark oil, dried and evaporated *in vacuo*. The residue was distributed between water and ether, and the ethereal layer was dried and acidified with ethanolic hydrogen chloride. The precipitated hydrochloride (0·91 g.) was worked up as in the previous experiment, affording *N*-thiobenzoylbenzamidine (0·53 g., 25%), m. p. 73—74°, undepressed by the material prepared from thiobenzamide and phenyl cyanide.

In the same way *N*-benzoyl-*p*-methoxybenzamidine yielded p-*methoxy*-N-*thiobenzoylbenzamidine* (48·5%), red leaflets (from benzene–light petroleum), m. p. 116—117° (Found : C, 66·9; H, 5·1; N, 10·4 $C_{15}H_{14}ON_2S$ requires C, 66·7; H, 5·2; N, 10·4%).

N-Benzoyl-*p*-chlorobenzamidine similarly yielded p-*chloro*-N-*thiobenzoylbenzamidine* (26%), red needles (from benzene or chloroform), m. p. 146—147° (Found : C, 61·3; H, 4·15; N, 9·9. $C_{14}H_{11}N_2ClS$ requires C, 60·8; H, 4·0; N, 10·2%).

Attempted Preparation of p-*Methylsulphonyl*-N-*thiobenzoylbenzamidine*.—*N*-Benzoyl-*p*-methylsulphonylbenzamidinium chloride (2·03 g.; m. p. 210—212°) appeared to react only partially with phosphorus pentachloride in chloroform. After 30 minutes, the insoluble residue (0·66 g.) was filtered off. It dissolved in water and the solution soon crystallised. The product (0·36 g.; m. p. 170—172°) crystallised from ethanol in glistening plates, m. p. 171—172° (Found : C, 59·8; H, 4·5; N, 4·7. $C_{15}H_{13}O_4NS$ requires C, 59·4; H, 4·3; N, 4·6%). It was soluble in dilute sodium hydroxide solution and was reprecipitated by acetic acid. The same material was obtained by crystallisation of *N*-benzoyl-*p*-methylsulphonylbenzamidinium chloride from 95% ethanol. The compound is therefore *benzoyl*-p-*methylsulphonylbenzoylamine*.

The chloroform-soluble material was processed in the usual manner. Treatment with a chloroform solution of triethylamine saturated with hydrogen sulphide caused an immediate red colouration but nothing corresponding to the desired product could be obtained by working-up the mixture after 90 minutes. When the solution was kept, the red colour changed to deep yellow and yellow crystals separated. These were *p*-methylsulphonylthiobenzamide (0·40 g.), m. p. 214—215° (decomp.) after crystallisation from butanol and undepressed by authentic material (see below). Addition of water to the chloroform mother-liquor afforded a further

quantity (0·20 g.) of *p*-methylsulphonylthiobenzamide. The chloroform layer was evaporated to dryness and the residue extracted with ether. Evaporation of the extract and crystallisation of the residue from benzene gave thiobenzamide (0·19 g.), m. p. 113—114° undepressed by authentic material.

p-*Methylsulphonylthiobenzamide.*—*p*-Methylsulphonylphenyl cyanide (0·90 g.) was dissolved in a mixture of chloroform (15 c.c.) and triethylamine (1·5 g., 3 mols.) and saturated at 0° with a stream of dry hydrogen sulphide. The solution rapidly became yellow and solid separated. After 6 hours the solid (0·96 g.; m. p. 212—214°) was isolated. Two recrystallisations from butanol furnished the pure *thioamide* as pale yellow needles, m. p. 217—218° (decomp.) (Found: N, 6·5. $C_8H_9O_2NS_2$ requires N, 6·5%).

1 : 2 : 4-*Triphenyl*-1 : 3 : 5-*triazapenta*-1 : 3-*diene* (II; R = R′ = H, R″ = Ph).—A mixture of *N*-thiobenzoylbenzamidine (0·48 g.), mercuric oxide (1·81 g.), aniline (1·0 g.), and absolute ethanol (10 c.c.) was shaken until the supernatant liquid had changed from red to pale yellow (*ca.* 1 hour) and the mercuric oxide had become dark brown. The reaction mixture was filtered and the filtrate was evaporated *in vacuo*. Trituration of the partly crystalline residue with ether–light petroleum afforded 1 : 2 : 4-triphenyl-1 : 3 : 5-triazapenta-1 : 3-diene (0·40 g.; m. p. *ca.* 145°). Recrystallisation from ether and from ethanol gave the pure product, m. p. 146—147° undepressed by material prepared from *N*-phenylbenzimidoyl chloride and benzamidine (Part I, *loc. cit.*).

Fission of N-*Thiobenzoylbenzamidine.*—(a) *With ammonia.* *N*-Thiobenzoylbenzamidine (0·12 g.) was dissolved in 2N-absolute ethanolic ammonia (3 c.c.). The red colour had been completely discharged after 30 minutes. Evaporation *in vacuo* and trituration of the residue with water (2 c.c.) gave thiobenzamide (0·06 g., 88%), m. p. and mixed m. p. 116—117°. Evaporation of the mother-liquor and treatment with picric acid in benzene afforded benzamidinium picrate (0·13 g., 74%), m. p. and mixed m. p. 238—239°.

In the presence of mercuric oxide, mercuriammonium chloride, or lead hydroxide, there were isolated in addition cyaphenin, 3 : 5-diphenyl-1 : 2 : 4-thiadiazole, needles (from methanol), m. p. 88—89° (Found: C, 70·4; H, 4·3; N, 11·9. Calc. for $C_{14}H_{10}N_2S$: C, 70·6; H, 4·2; N, 11·8%), and an unidentified sulphur-containing *picrate*, yellow needles (from ethanol), m. p. 187—188°) (Found: C, 54·1, 53·7; H, 4·1, 4·2; N, 16·9, 16·8. $C_{20}H_{20}N_4S,C_6H_3O_7N_3$ requires C, 54·0; H, 4·0; N, 17·0%).

(b) *With benzylamine.* A solution of *N*-thiobenzoylbenzamidine (3·84 g.) and benzylamine (1·71 g., 1 mol.) in dry ether (40 c.c.) became almost colourless after a few hours. A small amount of flocculent insoluble precipitate was filtered off and the filtrate shaken with N-hydrochloric acid (20 c.c.). The aqueous layer was made alkaline to Titan-yellow with lithium hydroxide, and the liberated oil (3·16 g.) isolated with ether. Treatment of this oil with ethanolic picric acid gave *N*-benzylbenzamidinium picrate (5·50 g., 78%), m. p. and mixed m. p. 168—170°. Concentration of the ethanolic mother-liquor afforded a small quantity (0·07 g.) of benzamidinium picrate, m. p. and mixed m. p. 238—239° after recrystallisation from *iso*propanol. A further quantity (0·12 g.) was obtained by addition of picric acid to the aqueous lithium hydroxide layer and acidification with acetic acid.

The original ethereal layer was evaporated and the residue extracted with boiling water (2 × 20 c.c.). The extracts on cooling afforded thiobenzamide (1·28 g., 58·5%), m. p. and mixed m. p. 115—116°. The insoluble residue, crystallised from 50% ethanol (20 c.c.), gave *N*-benzylthiobenzamide as plates (0·12 g.), m. p. and mixed m. p. 84—85°.

2 : 4-*Diphenyl*-1 : 3 : 5-*triazapenta*-1 : 3-*diene* (III*a*).—*N*-Thiobenzoylbenzamidine (4·8 g.) and benzamidine (2·4 g., 1 mol.) were dissolved in dry ether (95 c.c.), and the solution kept overnight. The red colour had almost disappeared and a white solid had separated. This was collected and identified by m. p. and mixed m. p. as cyaphenin (1·52 g.). A further small amount of cyaphenin (0·18 g.) separated on cooling the solution to −70°. 5N-Ethanolic hydrogen chloride was added to the filtrate until it was acid to moist Congo-red paper, and the precipitated solid (3·55 g.) collected. Evaporation of the filtrate and crystallisation of the residue from benzene afforded thiobenzamide (1·0 g.). The precipitated solid was triturated with water (13 c.c.) to remove benzamidinium chloride, and the undissolved solid was collected and washed with water (5 c.c.). The crude dry product (2·81 g.) was dissolved in warm absolute ethanol (40 c.c.) and filtered. Addition of acetone (80 c.c.) gave 2 : 4-*diphenyl*-1 : 3 : 5-*triazapenta*-1 : 3-*diene hydrochloride hemihydrate* as feathery needles (2·16 g.), m. p. 204—206° to a turbid liquid clearing at 230° [Found: C, 62·5; H, 5·6; N, 15·5; Cl, 13·8; H_2O (Karl Fischer), 3·4. $C_{14}H_{14}N_3Cl,0{\cdot}5H_2O$ requires C, 62·5; H, 5·6; N, 15·6; Cl, 13·2; H_2O, 3·3%]. The m. p. behaviour was unaltered by further crystallisation. The compound melted completely

when placed in a bath at 215°, then became turbid, and again cleared at 230°. A portion (100 mg.), fused at 205—210°, gave a semi-solid melt which smelt strongly of phenyl cyanide. Extraction of the melt with cold methanol afforded cyaphenin (22 mg.) as insoluble residue. The methanol extract was evaporated to dryness and the residue dissolved in water. Addition of lithium picrate solution gave benzamidinium picrate (107 mg., 82%).

The hydrochloride had a low solubility in water (*ca.* 1 in 250). The neutral saturated solution slowly deposited cyaphenin as small needles. Addition of a drop of 5N-hydrochloric acid to the saturated solution caused immediate reprecipitation of the hydrochloride as fine needles. Overnight these disappeared and were replaced by stouter, opaque needles, m. p. 146—147°, identified by mixed m. p. as dibenzoylamine.

Addition of an ethereal picric acid solution instead of ethanolic hydrogen chloride to the reaction mixture afforded the *picrate* as yellow prisms, m. p. 188° to a turbid melt clearing at 230°, after crystallisation from acetone–ether (Found: C, 52·7; H, 3·6; N, 18·8. $C_{14}H_{13}N_3,C_6H_3O_7N_3$ requires C, 53·0; H, 3·55; N, 18·6%). The picrate melted completely if placed in a bath at 205°, then became turbid, and cleared at 230°. Colourless needles sublimed up the m. p. tube during heating, and sublimation of the picrate at 200°/2 mm. gave pure cyaphenin. Under the same conditions benzamidinium picrate sublimes unchanged.

The free base, prepared by shaking the hydrochloride with ether and dilute sodium hydrogen carbonate solution, was an oil which gradually deposited cyaphenin over a period of days.

2 : 4-*Di*-p-*methoxyphenyl*-1 : 3 : 5-*triazapenta*-1 : 3-*diene* (III*b*).—A solution of *p*-methoxybenzamidine (0·56 g.) and *p*-methoxy-*N*-thiobenzoylbenzamidine (1·0 g.) in ether (250 c.c.) became almost colourless after 40 hours. Precipitation with ethanolic hydrogen chloride afforded a hygroscopic solid (1·2 g.). This was triturated with water (7 c.c.), and then washed with acetone, and the resulting pale yellow solid (0·62 g.) crystallised from methanol–acetone. 2 : 4-*Di*-p-*methoxyphenyl*-1 : 3 : 5-*triazapenta*-1 : 3-*diene hydrochloride monohydrate* (0·34 g.) separated in colourless prisms, m. p. 176—178° to a clear melt (Found: C, 57·15; H, 6·0; N, 12·4; Cl, 10·7. $C_{16}H_{18}O_2N_3Cl,H_2O$ requires C, 56·9; H, 5·9; N, 12·4; Cl, 10·5%).

2 : 4-*Di*-p-*chlorophenyl*-1 : 3 : 5-*triazapenta*-1 : 3-*diene* (III*c*).—In the same way *p*-chlorobenzamidine (0·47 g.) and *p*-chloro-*N*-thiobenzoylbenzamidine (0·82 g.) in ether (200 c.c.) afforded 2 : 4-*di*-p-*chlorophenyl*-1 : 3 : 5-*triazapenta*-1 : 3-*diene hydrochloride hemihydrate* (0·48 g.), feathery needles (from methanol–acetone), m. p. 214—215° to a turbid melt not clearing below 260°. Repeated recrystallisation from methanol–acetone raised the m. p. to 220—221° (Found: C, 49·8; H, 3·9; N, 12·6. $C_{14}H_{12}N_3Cl_3,0{\cdot}5H_2O$ requires C, 49·7; H, 3·85; N, 12·4%)

2-p-*Methoxyphenyl*-4-*phenyl*-1 : 3 : 5-*triazapenta*-1 : 3-*diene* (III*d*).—A solution of benzamidine (0·44 g.) and *p*-methoxy-*N*-thiobenzoylbenzamidine (1·0 g.) in ether (25 c.c.) was kept for 24 hours. The almost colourless solution was filtered from a little solid and acidified with 5N-ethanolic hydrogen chloride (1 c.c.). The sticky precipitate was separated by decantation, then triturated with water (5 c.c.), and the residual solid washed with acetone. Crystallisation from methanol–ether gave the *hydrochloride monohydrate* as fluffy needles (0·115 g.) which melted if placed in a bath at 140° but on slow heating did not melt till 163—165°, then giving a turbid liquid (Found: C, 58·9; H, 5·85; N, 13·8. $C_{15}H_{16}ON_3Cl,H_2O$ requires C, 58·5; H, 5·85; N, 13·7%).

Fission of N-*Thiobenzoylbenzamidine with* N-*Phenylbenzamidine.*—No reaction occurred between *N*-thiobenzoylbenzamidine and *N*-phenylbenzamidine in ether at room temperature over a period of several days. Accordingly, a solution of the former (0·96 g.) and the latter (0·76 g.) in benzene (8 c.c.) was heated under reflux until it was colourless (60 hours). Cyaphenin (0·45 g.) crystallised on cooling. The benzene filtrate was shaken with N-hydrochloric acid (5 c.c.), and the hydrochloride which separated was collected. This material (20 mg.; m. p. *ca.* 250°) was converted into the free base with aqueous sodium hydroxide, isolated with ether, and crystallised from methanol. It separated in needles, m. p. 181—182° not depressed by 1 : 2 : 4 : 5-tetraphenyl-1 : 3 : 5-triazapenta-1 : 3-diene (Part I; *loc. cit.*).

Fission of N-*Benzoylbenzamidine.*—(a) *With ethanol.* *N*-Benzoylbenzamidine (0·50 g.) was heated under reflux for 24 hours with absolute ethanol (5 c.c.). The solid which separated during heating was identified by m. p. and mixed m. p. as cyaphenin (0·10 g.). The filtrate was evaporated and the residue shaken with ether (3 c.c.) and 2N-hydrochloric acid (2 c.c.). Basification of the aqueous acid layer gave recovered *N*-benzoylbenzamidine (0·21 g.), m. p. 102—103° after crystallisation from light petroleum. Evaporation of the ethereal layer afforded a small quantity of liquid which distilled completely at 212—230° (bath-temp.) without leaving any residue of benzamide and was identified as ethyl benzoate by conversion into benzanilide, m. p. and mixed m. p. 160°.

(b) *With ammonia.* A solution of *N*-benzoylbenzamidine (0·50 g.) in 2N-ethanolic ammonia (5 c.c.) was kept for 7 days at room temperature and then evaporated *in vacuo*. The residue dissolved completely in 2N-hydrochloric acid (2 c.c.). Basification afforded unchanged *N*-benzoylbenzamidine (0·27 g.). Addition of picric acid to the mother-liquor gave a crude picrate, repeated crystallisation of which afforded benzamidinium picrate (0·13 g.), m. p. and mixed m. p. 237—238°.

(c) *With benzylamine.* A solution of *N*-benzoylbenzamidine (0·90 g.) in absolute ethanol (10 c.c.) and benzylamine (0·43 g., 1 mol.) was kept for 7 days at room temperature and then evaporated *in vacuo*, finally over sulphuric acid. The residue was shaken with ether and 2N-hydrochloric acid. The ethereal layer was evaporated and the residue (0·08 g.) crystallised from benzene, affording benzamide (0·03 g.), m. p. and mixed m. p. 126—127°. Evaporation of the benzene mother-liquor and crystallisation of the residue from aqueous ethanol gave *N*-benzylbenzamide (0·02 g.), m. p. 103—104° not depressed by authentic *N*-benzylbenzamide, m. p. 104—105°. The hydrochloric acid layer was made alkaline to Titan-yellow with solid lithium hydroxide, and the precipitated oil isolated with benzene. Treatment with alcoholic picric acid and recrystallisation of the crude product from ethanol gave *N*-benzylbenzamidinium picrate (0·71 g., 40·5%), m. p. and mixed m. p. 168—169°. The aqueous lithium hydroxide mother-liquor, neutralised with picric acid, afforded benzamidinium picrate (0·03 g.), m. p. 236—237° undepressed by authentic material

(d) *With aniline at* 180°. A mixture of *N*-benzoylbenzamidine (4·5 g.) and aniline (1·9 g., 1 mol.) was heated gradually to 180°. Vigorous evolution of ammonia commenced at *ca.* 170° but soon slackened and appeared complete after 2 hours. The dark melt solidified on cooling and was extracted with ether (20 c.c.). The insoluble residue (2·41 g.) was separated by differential solubility in ethanol into cyaphenin (0·52 g.), m. p. and mixed m. p. 233—234°, and benzamide (1·75 g., 72%), m. p. and mixed m. p. 125—126°.

The ethereal extract was evaporated and the residual grey solid (1·52 g.), freed from traces of aniline *in vacuo* over sulphuric acid, was extracted with N-hydrochloric acid (10 c.c.). Crystallisation of the insoluble residue from 70% ethanol afforded benzanilide (0·12 g., 3%), m. p. and mixed m. p. 160—162°. The acid extract quickly deposited prisms (0·34 g., 5·5%), m. p. 289—292° after crystallisation from aqueous hydrochloric acid. Sachs and Bry (*Ber.*, 1901, **34**, 122) record m. p. 286° for *NN'*-diphenylbenzamidinium chloride, and the identity was confirmed by conversion into the free base, m. p. 146—147° undepressed by *NN'*-diphenylbenzamidine. The hydrochloric acid mother-liquor, basified with aqueous sodium hydroxide, afforded a solid which after two crystallisations from light petroleum melted at 111—113° (0·30 g., 7·5%), undepressed by *N*-phenylbenzamidine. Its identity was confirmed by conversion into its picrate, m. p. 148—149° undepressed by *N*-phenylbenzamidinium picrate, m. p. 148·5°.

N-Benzoyl-N-phenylbenzamidine.—A suspension of *N*-phenylbenzamidine (9·8 g.) in pure chloroform (100 c.c.) and triethylamine (6·06 g., 1·02 mols.) was cooled, and redistilled benzoyl chloride (7·03 g., 1 mol.) added with stirring at 0—1°. When the solid had gone into solution, the reaction mixture was washed with an excess of ice-cold dilute sodium carbonate solution, and the chloroform layer was dried (Na_2SO_4) and evaporated *in vacuo*. The gummy residue was triturated with ether (100 c.c.), and the resulting solid (16·0 g.; m. p. 99—100°) was recrystallised by dissolving it in cold chloroform (26 c.c.) and adding ether (100 c.c.). The product separated in needles (5·55 g.), which partly melted to a turbid liquid at 102—104°, resolidified, and then remelted at 139—140°. It melted completely when placed in a bath at 110°. Repeated recrystallisation gave material melting initially to a turbid melt at 104—105° (Found: N, 9·5. Calc. for $C_{20}H_{16}ON_2$: N, 9·3%). In agreement with Wheeler *et al.* (*loc. cit.*) it gave benzanilide on hydrolysis with aqueous ethanolic hydrochloric acid and isomerised to *N'*-benzoyl-*N*-phenylbenzamidine when crystallised from ethanol.

Reaction of N'-Benzoyl-N-phenylbenzamidine.—(a) *With ammonia.* *N'*-Benzoyl-*N*-phenylbenzamidine (0·60 g.) was shaken with 2N-ethanolic ammonia (10 c.c.) until all was in solution. After 24 hours the solution was evaporated *in vacuo*. The residue dissolved completely in ether (15 c.c.), showing the absence of more than traces of starting material. The ethereal solution was extracted with N-hydrochloric acid (3 c.c.). Evaporation of the ethereal layer and crystallisation of the residue from benzene afforded benzamide (0·045 g., 19%), m. p. and mixed m. p. 125—126°. The acid layer was basified with 5N-sodium hydroxide, and the oil (0·40 g.) isolated with ether. Conversion into the picrate by saturated ethereal picric acid solution gave an immediate precipitate (0·29 g., 32%), m. p. 198—200°, identified by mixed m. p. as *N*-benzoylbenzamidinium picrate. When kept, the ethereal mother-liquor deposited *N*-phenylbenzamidinium picrate (0·25 g., 30%).

(b) *With benzylamine.* A solution of *N'*-benzoyl-*N*-phenylbenzamidine (0·90 g.) and benzylamine (0·31 c.c.) in absolute ethanol was heated under reflux for 12 hours. The ethanol was blown off in a stream of air, and the residue triturated with 2N-acetic acid and ether (5 c.c. of each). The mixture soon deposited prisms (0·52 g., 55·5%), recrystallisation of which from ethanol afforded *N'*-benzoyl-*N*-benzylbenzamidine, m. p. 149—150° (Found : C, 80·7; H, 5·4; N, 8·9. Calc. for $C_{21}H_{18}ON_2$: C, 80·3; H, 5·7; N, 8·9%). Beckmann and Sandel (*loc. cit.*) record m. p. 147° for this compound. Basification of the aqueous acetic acid layer gave *N*-phenylbenzamidine (0·10 g., 17%), m. p. and mixed m. p. 114—115°.

The same product was formed slowly at room temperature.

Reaction of N-*Benzoyl*-N-*phenylbenzamidine with Ammonia.*—A solution of *N*-benzoyl-*N*-phenylbenzamidine (3·0 g.) in 2N-ethanolic ammonia was kept at room temperature. No unchanged material could be detected after 24 hours. The residue after evaporation *in vacuo* was shaken with ether and water. The insoluble solid (1·21 g.) was identified as benzanilide. The aqueous layer was alkaline to brilliant-yellow. Acidification with saturated ethanolic picric acid afforded benzamidinium picrate (0·10 g., 2%), m. p. 236—237° raised by authentic material. Basification of the hydrochloric acid extract of the ethereal layer gave a crude base (0·21 g.), separated by conversion by saturated ethereal picric acid into *N*-benzoylbenzamidinium picrate (0·09 g., 2%), m. p. and mixed m. p. 199—200° after recrystallisation from ethanol, and *N*-phenylbenzamidinium picrate (0·20 g., 5%), m. p. and mixed m. p. 148—149°. The original ethereal layer on evaporation afforded an oily solid, separated by extraction with light petroleum into phenyl cyanide (0·12 g.), characterised as its *m*-nitro-derivative, and crude benzanilide (0·13 g.). The total yield of benzanilide amounted to 68%. Extraction of the combined evaporated mother-liquors with hot benzene did not yield any benzamide.

NN'-*Dibenzoyl*-N-*phenylbenzamidine.*—Benzoyl chloride (3·01 g., 1 mol.) was added to a suspension of *N*-phenylbenzamidine (4·2 g., 1 mol.) in dry ether (100 c.c.), and the mixture shaken occasionally during 1 hour. The precipitate of *N*-phenylbenzamidinium chloride was filtered off and the filtrate evaporated to dryness. Crystallisation of the residue from ethanol (15 c.c.) gave NN'-*dibenzoyl*-N-*phenylbenzamidine* (1·06 g.) as plates, m. p. 141—142°, considerably depressed by *N'*-benzoyl-*N*-phenylbenzamidine (Found : N, 6·8. $C_{27}H_{20}O_2N_2$ requires N, 6·9%).

A solution of the above compound (0·50 g.) in 5N-hydrochloric acid (10 c.c.) and ethanol (5 c.c.) was shaken overnight and the solid (0·45 g.) collected. Extraction with dilute sodium hydroxide solution gave benzanilide (0·13 g.), m. p. and mixed m. p. 160—161°. Acidification of the alkaline extract gave dibenzoylamine (0·10 g.), m. p. and mixed m. p. 145—147°.

The dibenzoyl compound (0·5 g.) was dissolved in 2N-ethanolic ammonia (10 c.c.) by shaking for 90 minutes and the solution kept overnight. Evaporation *in vacuo* and trituration of the residue with N-hydrochloric acid (5 c.c.) afforded, after crystallisation from ethanol, benzanilide (0·13 g.), m. p. and mixed m. p. 160—161°. The acid filtrate was basified with sodium hydroxide, and the resulting gummy precipitate isolated with ether. Conversion into the picrate afforded, after crystallisation from ethanol, yellow needles (0·10 g.), m. p. 202—203° identified by mixed m. p. as *N*-benzoylbenzamidinium picrate.

Attempted Reaction of N-*Benzenesulphonylbenzamidine with Ammonia.*—*N*-Benzenesulphonylbenzamidine was satisfactorily prepared by Barber's method (*J.*, 1943, 101) for *p*-nitrobenzenesulphonylbenzamidine. The compound (0·5 g.) was shaken with 2N-ethanolic ammonia (10 c.c.) for 3 days. Unchanged material (0·44 g.) was recovered. No trace of benzamidine or benzenesulphonamide could be detected in the ethanolic mother-liquor.

Condensation of Benzamidine and Ethyl Benzimidate.—(*a*) Benzamidine (2·53 g.) was dissolved in dry ether (154 c.c.), and ethyl benzimidate (3·14 g., 1 mol.) added. After 5 days an aliquot (20 c.c.) was made acid to moist Congo-red paper by the addition of 5N-ethanolic hydrogen chloride (2 c.c.). The precipitate (0·85 g.) dissolved readily in cold water (2 c.c.), indicating the absence of more than traces of the diamidide (III*a*) hydrochloride.

The remaining ethereal solution was evaporated and the residue heated at 100° for 24 hours. On cooling and dilution with ether (40 c.c.), a small amount of solid (0·015 g.; m. p. 233—234°) separated and was identified as cyaphenin. The filtrate was acidified with 5N-ethanolic hydrogen chloride (4 c.c.) and the precipitated solid collected. Trituration with water (3·0 c.c.) left an insoluble residue (0·26 g.) which crystallised from ethanol–acetone in fine needles (0·13 g., 2·6%), showing the characteristic m. p. behaviour of the diamidide (III*a*) hydrochloride.

(*b*) A solution of benzamidine (2·39 g.) and ethyl benzimidate (3·26 g., 1·1 mols.) in dry ether (500 c.c.) was heated under reflux and the condensate returned to the flask through a Soxhlet extractor containing sodium wire. After 4 days the sodium remained practically

unattacked and working-up as above of an aliquot (50 c.c.) indicated the absence of more than traces of diamidide (III*a*) hydrochloride.

The ether was therefore replaced by dry benzene and heating under reflux continued for 3 days. The benzene solution was cooled, cyaphenin (0·49 g.) filtered off, and the filtrate acidified with 5N-ethanolic hydrogen chloride (4·5 c.c.). Only a slight precipitate formed. The benzene was accordingly evaporated *in vacuo* and the residue triturated with ether (30 c.c.). The residual pale-yellow solid (2·37 g.) was triturated with water (4 c.c.), and the residue extracted with cold methanol (10 c.c.). The insoluble residue (0·84 g.; m. p. 233—234°) was identified as cyaphenin. The methanol filtrate was evaporated *in vacuo*. Crystallisation of the residue from ethanol–acetone afforded the diamidide (III*a*) hydrochloride (0·05 g., 1·05%).

Pyrolysis of Benzamidine in Benzene.—A solution of benzamidine (1·27 g.) in dry benzene (270 c.c.) was heated under reflux for 3 days, ammonia being evolved throughout. Worked up as above, the reaction yielded diamidide (III*a*) hydrochloride in 0·35% yield.

The author gratefully acknowledges his indebtedness to Dr. W. F. Short for his interest and for helpful discussion, and to Mr. P. Mabbitt for technical assistance. Thanks are also offered to Mr. L. Brealey for spectroscopic data and to Mrs. Weston, Miss Coleman, and Mr. Elsom for the analyses.

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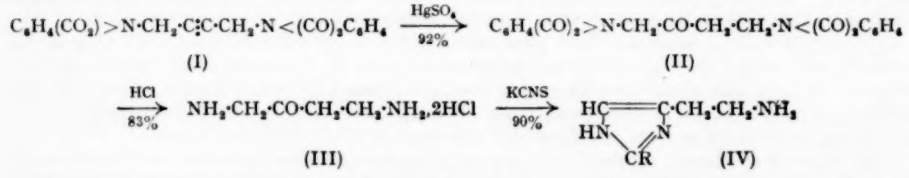
44. *A Synthesis of Histamine from But-2-yne-1 : 4-diol.*

By M. M. FRASER and R. A. RAPHAEL.

1 : 4-Diphthalimidobut-2-yne (I), prepared by the interaction of 1 : 4-dichlorobut-2-yne and potassium phthalimide, undergoes smooth hydration to 1 : 4-diphthalimidobutan-2-one (II) which is readily hydrolysed to the parent diamine dihydrochloride (III). Condensation of (III) with potassium thiocyanate and subsequent oxidation furnishes histamine (IV; R = H).

ALTHOUGH the synthesis of the allergenic agent histamine (4-2′-aminoethylglyoxaline) (IV; R = H) was first realised by Windaus and Vogt (*Ber.*, 1907, **40**, 3691) the first practicable preparation was devised by Pyman employing an eight-stage synthesis from citric acid (*J.*, 1911, **99**, 668; cf. Koessler and Hanke, *J. Amer. Chem. Soc.*, 1918, **40**, 1716). The recent availability of one of the intermediates (4-hydroxymethylglyoxaline) from fructose (Totter and Darby, *Org. Synth.*, 1944, **24**, 64) has shortened this reaction sequence but even with this improvement the synthesis is tedious and the overall yield is only moderate. The investigation of new synthetic methods has up to now revealed no more advantageous route (Akabori and Numano, *Bull. Chem. Soc., Japan*, 1936, **11**, 214; Dankova, Sidorova, and Preobrashenski, *J. Gen. Chem., U.S.S.R.*, 1945, **15**, 674).



The recent commercial availability of but-2-yne-1 : 4-diol suggested a new approach to the compound. This glycol was converted by thionyl chloride in high yield into 1 : 4-dichlorobut-2-yne (Johnson, *J.*, 1946, 1009). Reaction of the dichloride with a solution of potassium phthalimide in dimethylformamide (Sheehan and Bolhofer, *J. Amer. Chem. Soc.*, 1950, **72**, 2786) furnished an excellent yield (91%) of the corresponding diphthalimido-compound (I). Under the influence of mercuric sulphate catalyst in acetic acid the triple bond of (I) was smoothly hydrated, 1 : 4-diphthalimidobutan-2-one (II)

being obtained in nearly theoretical yield; hydrolysis with hydrochloric acid then produced 1 : 4-diaminobutan-2-one dihydrochloride (III). This compound has already been prepared by Pyman (*J.*, 1930, 98) by degradation of histamine and was reconverted by him into the parent compound. In an analogous but modified manner (III) was condensed with potassium thiocyanate to give, in almost quantitative yield, the hydrochloride of 2-mercaptohistamine (IV; R = SH), oxidation of which with ferric chloride, followed by treatment with picric acid, furnished the dipicrate of histamine (IV; R = H).

The facility with which the above reactions proceed is demonstrated by the high overall yield (41%) of histamine dipicrate from but-2-yne-1 : 4-diol. The sequence is obviously applicable to other similarly constituted acetylenic glycols to furnish analogues of histamine which may possibly possess anti-histaminic properties; this is now being investigated.

Experimental

1 : 4-*Dichlorobut-2-yne.*—This was prepared from the glycol by the action of 2·4 mols. of thionyl chloride in pyridine as described by Johnson (*loc. cit.*), yields similar to his being obtained. In some runs a small amount of 4-*chlorobut-2-yn-1-ol*, b. p. 87—88°/12 mm., 105—107°/25 mm., n_D^{19} 1·5007, was isolated (Found: C, 45·3; H, 4·9. C_4H_5OCl requires C, 45·95; H, 4·8%), the *phenylurethane* of which crystallised from light petroleum (b. p. 60—80°) in needles, m. p. 77—78° (Found: N, 6·2. $C_{11}H_{10}O_2NCl$ requires N, 6·25%). This chloro-alcohol constitutes the main product when only 1 mol. of thionyl chloride is employed in the reaction (Dr. M. C. Whiting, personal communication).

1 : 4-*Diphthalimidobut-2-yne.*—To a stirred solution of potassium phthalimide (31 g.) in dimethylformamide (130 c.c.) was added slowly 1 : 4-dichlorobut-2-yne (10·2 g.), and the mixture heated by steam for 7 hours. After being cooled the reaction mixture was treated with water (200 c.c.), and the precipitated solid (26 g., 91%), m. p. 277—279°, filtered off, washed with water, and dried. The *diphthalimido*-compound crystallised from acetic acid in pale yellow prisms, m. p. 281—282° (Found: N, 8·3. $C_{20}H_{12}O_4N_2$ requires N, 8·15%).

1 : 4-*Diphthalimidobutan-2-one.*—To a solution of 1 : 4-diphthalimidobut-2-yne (3 g.) in 90% acetic acid (140 c.c.) was added mercuric acetate (750 mg.), followed by concentrated sulphuric acid (0·5 c.c.), and the mixture heated under reflux for 4 hours. Water (200 c.c.) was added to the resulting solution and, after an hour's standing, the precipitated solid was filtered off and washed with water. The *diphthalimido-ketone* (2·9 g., 92%) crystallised from acetic acid in white plates, m. p. 248—249° (Found: C, 66·3; H, 4·3; N, 7·5. $C_{20}H_{14}O_5N_2$ requires C, 66·3; H, 3·9; N, 7·7%). Treatment with hydroxylamine hydrochloride in pyridine gave the corresponding *oxime*, crystallising from alcohol in needles, m. p. 225—226° (Found: N, 10·65. $C_{20}H_{15}O_5N_3$ requires N, 11·1%).

1 : 4-*Diaminobutan-2-one Dihydrochloride.*—A mixture of the foregoing ketone (8·1 g.), acetic acid (150 c.c.), and concentrated hydrochloric acid (150 c.c.) was heated under reflux for 36 hours with further additions (each 40 c.c.) of the 1 : 1 acetic acid–hydrochloric acid after 4 and 24 hours. The resulting solution was treated with charcoal, filtered, and evaporated to small bulk (60 c.c.). On being cooled the solution deposited phthalic acid (6·1 g., 82%) which was filtered off and washed with a little water. The filtrate was evaporated under reduced pressure (20 c.c.), alcohol (100 c.c.) added, and the solution kept at 0°. The crystalline solid (3·15 g.) thus obtained was filtered off and the filtrate evaporated to obtain a further small quantity of the product. The combined yield was suspended in boiling alcohol, and hot water added slowly until dissolution was effected. On being cooled the solution deposited the diamino-ketone dihydrochloride (3·25 g., 83%) in elongated plates, m. p. 217° (decomp.) [Pyman, *loc. cit.*, gives m. p. 221° (decomp.; corr.)].

2-*Mercaptohistamine Hydrochloride.*—A solution of the diamino-ketone dihydrochloride (3·1 g.) and potassium thiocyanate (1·85 g.) in water (15 c.c.) was evaporated to a syrup which was then heated for 1 hour by steam. Just enough hot water was then added to dissolve the inorganic salts; when kept, the solution deposited pure 2-mercaptohistamine hydrochloride (0·94 g.) in long needles, m. p. 244—245° [Pyman, *loc. cit.* gives m. p. 248—249° (corr.)]. The mother-liquor was evaporated to dryness and extracted with three 25-c.c. portions of boiling methanol. Evaporation of the methanol in stages gave further quantities of the pure product (total yield, 2·84 g.; 90%).

Histamine Dipicrate.—A solution of 2-mercaptohistamine hydrochloride (155 mg.) and ferric chloride hexahydrate (1·39 g.) in water (10 c.c.) was heated under reflux for 1 hour. Enough solid sodium carbonate was then introduced to neutralise the mineral acid without

causing precipitation of iron carbonate, and a hot saturated aqueous solution of picric acid (395 mg.) added. After some time the crystalline precipitate of histamine dipicrate (384 mg., 80%) was filtered off and crystallised from aqueous ethanol whence it formed yellow prismatic plates, m. p. 234—235° (decomp.) [Pyman, *loc. cit.*, gives m. p. 238° (decomp.; corr.)].

Treatment of a portion of the dipicrate with concentrated hydrochloric acid and benzene, followed by removal of the benzene extract and evaporation of the aqueous layer, yielded histamine dihydrochloride as needles, m. p. 238—239° after crystallisation from methanol–ether [Koessler and Hanke, *loc. cit.*, give m. p. 244—246° (corr.)].

The authors are indebted to Professor J. W. Cook, F.R.S., for his interest in this work which was carried out during the tenure of a Chemical Society Research Grant.

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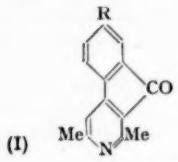
45. *New Syntheses of Heterocyclic Compounds. Part XIV.* Some New Derivatives of* 1 : 3-*Dimethyl-2-azafluorene.*

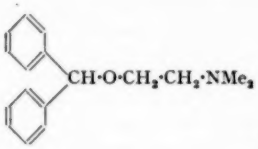
By B. N. FEITELSON and V. PETROW.

In the course of studies on the evaluation of the biological potentialities of 2-azafluorene, some 2-dialkylaminoalkyl ethers of 1 : 3-dimethyl-2-azafluorenol (III; R = H), and some *N*-substituted derivatives of 9-amino-1 : 3-dimethyl-2-azafluorene (V; R = H), have been prepared. Attempts to convert 1 : 3-dimethyl-2-azafluorene into the 9-carboxylic acid failed.

THE present investigation arose from the observation recorded by Kahn, Petrow, Rewald, and Sturgeon (*J.*, 1949, 2128) that 1 : 3-dimethyl-2-azafluorenone (I; R = H) shows more pronounced spasmolytic properties than does papaverine. As musculotropic function had not hitherto been associated with this compound, an evaluation of the wider potentialities of the basic ring system assumed importance. We have therefore prepared selected derivatives of 1 : 3-dimethyl-2-azafluorene and now report some compounds with structural analogies to drugs of established pharmacological potency.



(I)



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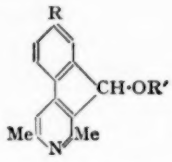

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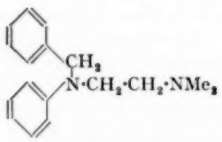

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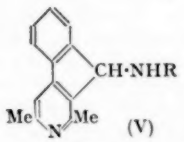

(V)

Initial experiments were directed towards the preparation of analogues of " Benadryl " (2-dimethylaminoethyl diphenylmethyl ether) (II), which has both antihistaminic and spasmolytic properties. 1 : 3-Dimethyl-2-azafluorenol (III; R = R′ = H) (Kahn *et al.*, *loc. cit.*) and 2-dimethylaminoethyl chloride, in the presence of sodamide, gave 1 : 3-dimethyl-9-2′-dimethylaminoethoxy-2-azafluorene (III; R = H, R′ = $CH_2 \cdot CH_2 \cdot NMe_2$) in low yield, as an oil which contained appreciable quantities of 1 : 3-dimethyl-2-azafluorenone, presumably formed from (III; R = R′ = H) by disproportionation (disproportionation was frequently encountered during the present investigation). The diethylamino-compound was similarly prepared. 7-Methoxy-1 : 3-dimethyl-2-azafluorenol, obtained by reduction of 1 : 3-dimethyl-7-methoxy-2-azafluorenone (Borsche and Hahn, *Annalen*,

* Part XIII, Courts and Petrow, *J.*, 1952, 1.

1939, **537**, 219) with zinc dust and ethanolic ammonia, furnished the corresponding 9-2′-diethylaminoethoxy-7-methoxy-1 : 3-dimethyl-2-azafluorene (III; R = OMe; R′ = $CH_2 \cdot CH_2 \cdot NEt_2$).

Attention was next directed to the preparation of compounds with structural features similar to the antihistaminic drug " Antergan " (IV). 9-Amino-1 : 3-dimethyl-2-azafluorene (V; R = H) (Kahn *et al.*, *loc. cit.*) with 2-diethylaminoethyl chloride in the presence of sodamide furnished 9-2′-diethylaminoethylamino-1 : 3-dimethyl-2-azafluorene (V; R = $CH_2 \cdot CH_2 \cdot NEt_2$) in low yield. Attempts to prepare other types, however, proved disappointing. *E.g.*, application of the Leuckart reaction to (I; R = H) failed. Reaction of (I; R = H) with formamide gave 9-formamido-1 : 3-dimethyl-2-azafluorene (V; R = CHO), but this could not be hydrolysed. Reductive acetylation of 7-methoxy-1 : 3-dimethyl-2-azafluorenone oxime led to 9-acetamido-7-methoxy-1 : 3-dimethyl-2-azafluorene in 54% yield, but its hydrolysis, when ultimately enforced, gave only a mixture of (I; R = H), some oil, and ammonium salts. The instability of the 9-amino-group in this series of compounds was further illustrated by the reaction of (V; R = H) with 1 : 5-dibromopentane, 1 : 3-dimethyl-2-azafluorenone being again the only product isolated.

Attempts to prepare 1 : 3-dimethyl-2-azafluorene-9-carboxylic acid, for conversion into an analogue of " Trasentin " (2-diethylaminoethyl diphenylacetate), failed: (i) 9-Chloro-1 : 3-dimethyl-2-azafluorene could not be prepared by methods used in the fluorene series (Schlenk and Bergmann, *Annalen*, 1928, **463**, 192; Courtot and Pierron, *Bull. Soc. chim.*, 1929, **45**, 290), but was readily obtained as the hydrochloride by treating 1 : 3-dimethyl-2-azafluorenol with thionyl chloride in chloroform. Careful treatment with aqueous ammonia gave the free base, which proved to be stable to boiling ethanol. The corresponding 9-bromofluorene hydrobromide was formed in high yield by refluxing the fluorenol with hydrobromic acid in acetic acid solution (cf. Ingold and Jessup, *J.*, 1929, 2357), and passed smoothly into the free bromo-compound on basification. The constitution assigned to these two compounds followed from their facile conversion into 1 : 3-dimethyl-9-piperidino-2-azafluorene. Reaction of 9-chloro- or 9-bromo-1 : 3-dimethyl-2-azafluorene with potassium cyanide in aqueous ethanol furnished an unidentified product, $C_{28}H_{23}N_3$, m. p. 202°; this was recovered unchanged after being heated with 50% sulphuric acid in aqueous or ethanolic solution, and attempted hydrolysis with alkali caused resinification. (ii) An alternative route to the 9-nitrile, whereby a 9-formylfluorene is converted into the oxime which is subsequently dehydrated (Von and Wagner, *J. Org. Chem.*, 1944, **9**, 162), was not successful in the present instance. Reaction of 1 : 3-dimethyl-2-azafluorene with ethyl formate in the presence of potassium methoxide gave a mixture from which an oxime could not be isolated. Hydrolysis with alkali led to the formation of a small quantity of (I; R = H). The 1 : 3-dimethyl-2-azafluorene was conveniently prepared by reduction of the fluorenone by the Huang-Minlon (*J. Amer. Chem. Soc.*, 1945, **67**, 1435) modification of the Wolf–Kishner method. (iii) 9-Chloro-1 : 3-dimethyl-2-azafluorene failed to react with magnesium. The corresponding bromo-compound, in contrast, readily did so under the usual experimental conditions. Carboxylation, as recommended by Gilman and Kirby (*J. Amer. Chem. Soc.*, 1926, **48**, 1735), failed to give the required acid. 9-Fluorenylmagnesium bromide gives only an 18% yield of the 9-carboxylic acid under these conditions (Campbell and Tucker, *J.*, 1949, 50). (iv) 1 : 3-Dimethyl-2-aza-9-fluorenyl-lithium was prepared by a halogen–metal interchange between the 9-bromo-base and butyl-lithium. Its carboxylation with solid carbon dioxide appeared to take place in the usual way, but the required 9-carboxylic acid could not be isolated. (v) Campbell and Tucker (*ibid.*, p. 2623) obtained fluorene-9-carboxylic acid in 75% yield by condensation of fluorene with methyl oxalate, followed by acid hydrolysis of the product. In our hands, 1 : 3-dimethyl-2-azafluorene gave a white crystalline product, $C_{16}H_{15}O_5N$, m. p. 216° (decomp.), of unknown constitution. As its formation may have involved reaction of one of the active methyl groups of the parent base with methyl oxalate, attempts were made to obtain 2-azafluorene for parallel study. Although we have improved Mills, Palmer, and Tomkinson's method (*J.*, 1924, 2365) for the preparation of 2-azafluorene, the yield was nevertheless too low to warrant condensation studies.

Reaction of 9-amino-1 : 3-dimethyl-2-azafluorene (V; R = H) with nicotinoyl chloride hydrochloride led to 1 : 3-dimethyl-9-nicotinamido-2-azafluorene. This may be regarded as a derivative of 3-nicotinamidomethylpyridine, which is claimed to produce a large and protracted lowering of the blood pressure (Roche Products Ltd., B.P. 639,246/1950). Treatment of 1 : 3-dimethyl-2-azafluorenone-4-carboxylic acid with 2-diethylaminoethyl chloride in *iso*propanol gave the corresponding ester.

Experimental

M. p.s are uncorrected.

1 : 3-*Dimethyl*-9-2′-*dimethylaminoethoxy*-2-*azafluorene*.—2-Dimethylaminoethyl chloride (from 7 g. of the hydrochloride) in benzene was added to sodamide (1·5 g.), 1 : 3-dimethyl-2-azafluorenol (7·03 g.), and benzene (80 ml.), and the mixture stirred under reflux for 4—4½ hours. After cooling somewhat and removal of precipitated sodium chloride, light petroleum was added and the solution set aside. 1 : 3-Dimethyl-2-azafluorenone (2·7 g.) separated and was removed. The mother-liquors were taken to dryness, the residue dissolved in absolute ethanol, and dry hydrogen chloride passed through the solution. 1 : 3-*Dimethyl*-9-2′-*dimethylaminoethoxy*-2-*azafluorene dihydrochloride dihydrate* was obtained (3·6 g.), having m. p. 266—267°, from alcohol (Found : C, 56·3; H, 6·9; N, 7·2; Cl, 18·2. $C_{18}H_{22}ON_2,2HCl,2H_2O$ requires C, 55·3; H, 7·2; N, 7·2; Cl, 18·1%). The *picrate* separated from ethanol–acetone in yellow crystals, m. p. 204° (Found : C, 48·2; H, 3·7; N, 14·6. $C_{18}H_{22}ON_2,2C_6H_3O_7N_3$ requires C, 48·7; H, 3·8; N, 15·1%).

9-2′-*Diethylaminoethoxy*-1 : 3-*dimethyl*-2-*azafluorene*.—2-Diethylaminoethyl chloride (5·7 g.), sodamide (1·9 g.), 1 : 3-dimethyl-2-azafluorenol (8·9 g.) and benzene (40 ml.) were heated under reflux with stirring for 4 hours, after which water (25 ml.) was added and the benzene layer extracted with 20% hydrochloric acid (25 ml.). Basification, followed by extraction with light petroleum, gave an oil which deposited 1 : 3-dimethyl-2-azafluorenone (500 mg.). The latter was removed, the residue dissolved in ethanol, and dry hydrogen chloride passed through the solution. 9-2′-*Diethylaminoethoxy*-1 : 3-*dimethyl*-2-*azafluorene dihydrochloride dihydrate* was obtained (22%) from ethanol, having m. p. 226—228° (Found : C, 58·7; H, 7·7; N, 6·5; Cl, 16·8. $C_{20}H_{26}ON_2,2HCl,2H_2O$ requires C, 58·4; H, 7·6; N, 6·5; Cl, 16·5%). The *picrate* formed needles, m. p. 169°, from ethanol–acetone (Found : C, 49·9; H, 4·2; N, 13·8. $C_{20}H_{26}ON_2,2C_6H_3O_7N_3$ requires C, 49·9; H, 4·2; N, 14·6%).

7-*Methoxy*-1 : 3-*dimethyl*-2-*azafluorenol*.—7-Methoxy-1 : 3-dimethyl-2-azafluorenone (11·5 g.), aqueous ammonia (250 ml.; *d* 0·880), zinc dust (80 g.), and ethanol (50 ml.) were heated under gentle reflux with stirring for 3 hours. The mixture was filtered hot and the solids were extracted 3 times with boiling ethanol. Concentration of the combined filtrates gave 7-*methoxy*-1 : 3-*dimethyl*-2-*azafluorenol* (10 g.), felted white needles (from benzene), m. p. 205° (Found : C, 74·4; H, 6·0; N, 6·0. $C_{15}H_{15}O_2N$ requires C, 74·7; H, 6·2; N, 5·8%). The *picrate* formed needles (from ethanol–acetone), m. p. 187° (Found : C, 53·8; H, 3·7; N, 11·9. $C_{15}H_{15}O_2N,C_6H_3O_7N_3$ requires C, 53·6; H, 3·8; N, 11·9%).

7-*Methoxy*-1 : 3-*dimethyl*-9-2′-*dimethylaminoethoxy*-2-*azafluorene dihydrochloride dihydrate* formed yellow crystals (15%), m. p. 250—251°, from ethanol–ether (Found : C, 54·1; H, 7·3; N, 7·2; Cl, 16·9. $C_{19}H_{24}O_2N_2,2HCl,2H_2O$ requires C, 54·2; H, 7·2; N, 6·7; Cl, 17·9%).

9-2′-*Diethylaminoethylamino*-1 : 3-*dimethyl*-2-*azafluorene*.—A mixture of 9-amino-1 : 3-dimethyl-2-azafluorene (5·2 g.) and sodamide (1·0 g.) in benzene (20 ml.) was gently heated with stirring under reflux for 1½ hours. 2-Diethylaminoethyl chloride (from 7 g. of the hydrochloride) in benzene was then added and heating continued for a further 6 hours. After removal of solids the liquors were taken to dryness, and the residue was dissolved in ethanol and treated with dry hydrogen chloride. 9-2′-*Diethylaminoethylamino*-1 : 3-*dimethyl*-2-*azafluorene trihydrochloride* was obtained (24%), having m. p. 280—281° (Found : C, 56·8; H, 7·0. $C_{20}H_{27}N_3,3HCl$ requires C, 57·3; H, 6·5%), from absolute ethanol. The *picrate* (from 2-ethoxyethanol) had m. p. 152° (Found : N, 16·8. $C_{20}H_{27}N_3,3C_6H_3O_7N_3$ requires N, 16·9%).

9-*Formamido*-1 : 3-*dimethyl*-2-*azafluorene*.—1 : 3-Dimethyl-2-azafluorenone (20 g.) and formamide (100 ml.) were heated under reflux for 30 minutes and the product was precipitated by addition to alkali. Crystallisation from benzene–light petroleum and finally from ethyl acetate furnished 9-*formamido*-1 : 3-*dimethyl*-2-*azafluorene*, needles, m. p. 236—237° (Found : C, 75·4; H, 5·9; N, 11·8. $C_{15}H_{14}ON_2$ requires C, 75·6; H, 5·9; N, 11·8%).

9-*Acetamido*-7-*methoxy*-1 : 3-*dimethyl*-2-*azafluorene*.—7-*Methoxy*-1 : 3-*dimethyl*-2-*azafluorenone*

oxime (83% yield) had m. p. 284° (from aqueous pyridine) (Found: C, 71·0; H, 5·5; N, 10·9. $C_{15}H_{14}O_2N_2$ requires C, 70·9; H, 5·5; N, 11·0%). This (5 g.), anhydrous sodium acetate (5 g.), and acetic anhydride (180 ml.) were heated under reflux, and treated with zinc dust (10 g.) portionwise, and refluxing was then continued for a further 1 hour. The mixture was filtered hot and poured into water (300 ml.), and the product precipitated by addition of aqueous ammonia. Purification from alcohol gave *9-acetamido-7-methoxy-1 : 3-dimethyl-2-azafluorene*, m. p. 278° (Found: C, 72·4; H, 6·4; N, 9·6. $C_{17}H_{18}O_2N_2$ requires C, 72·4; H, 6·4; N, 9·9%). The mother-liquors yielded 7-methoxy-1 : 3-dimethyl-2-azafluorenone, identified as the *picrate*, needles (from ethanol–acetone), m. p. 224—225° (Found: C, 54·4; H, 3·4; N, 11·9. $C_{17}H_{18}O_2N_2,C_6H_3O_7N_3$ requires C, 53·9; H, 3·4; N, 12·0%).

9-Chloro-1 : 3-dimethyl-2-azafluorene.—1 : 3-Dimethyl-2-azafluorenol (2·1 g.) was heated under reflux with excess of thionyl chloride for 2 hours. Removal of excess of thionyl chloride gave *9-chloro-1 : 3-dimethyl-2-azafluorene hydrochloride* (80%), m. p. >300° (from chloroform) (Found: C, 62·7; H, 4·8; N, 5·2; Cl, 26·3. $C_{14}H_{12}NCl,HCl$ requires C, 63·2; H, 4·9; N, 5·3; Cl, 26·7%). Treatment of an aqueous solution of the salt with dilute aqueous ammonia (to pH 7), followed by rapid cooling, gave *9-chloro-1 : 3-dimethyl-2-azafluorene* as needles (from light petroleum), m. p. 117° (Found: C, 74·1; H, 5·2; N, 5·8; Cl, 15·1. $C_{14}H_{12}NCl$ requires C, 73·2; H, 5·2; N, 6·1; Cl, 15·4%). This gave (I; R = H) on several hours' contact with aqueous ammonia.

9-Bromo-1 : 3-dimethyl-2-azafluorene.—1 : 3-Dimethyl-2-azafluorenol (65 g.), acetic acid (250 ml.), and a solution of hydrobromic acid in acetic acid (225 ml. of 50%) were heated under reflux for 2½ hours. Next morning *9-bromo-1 : 3-dimethyl-2-azafluorene hydrobromide* (94%) was collected; it had m. p. >300° (Found: C, 47·5; H, 3·7; N, 3·9; Br, 44·0. $C_{14}H_{12}NBr,HBr$ requires C, 47·3; H, 3·7; N, 3·9; Br, 45·0%). The free *base* formed needles from benzene–light petroleum, having m. p. 140—141° (Found: C, 60·9; H, 4·5; N, 5·3; Br, 28·3. $C_{14}H_{12}NBr$ requires C, 61·3; H, 4·4; N, 5·1; Br, 29·1%).

1 : 3-Dimethyl-9-piperidino-2-azafluorene.—(*a*) 9-Chloro-1 : 3-dimethyl-2-azafluorene hydrochloride (2·66 g.), piperidine (2·6 g.), and ethanol (10 ml.) were heated under reflux for 2 hours. The product, steam-distilled for removal of unchanged piperidine, was crystallised from light petroleum, to give *1 : 3-dimethyl-9-piperidino-2-azafluorene*, large octagonal prisms (50%), m. p. 120° (Found: N, 9·7. $C_{19}H_{22}N_2$ requires N, 10·1%). The *picrate*, from 2-ethoxyethanol, formed needles, m. p. 214° (Found: C, 51·0; H, 3·8; N, 14·7. $C_{19}H_{22}N_2,2C_6H_3O_7N_3$ requires C, 50·5; H, 3·8; N, 15·2%).

(*b*) The foregoing compounds were likewise prepared from 9-bromo-1 : 3-dimethyl-2-azafluorene.

(*c*) An attempt to prepare the piperidino-compound by heating 9-amino-1 : 3-dimethyl-2-azafluorene (2·1 g.) with 1 : 5-dibromopentane (2·5 g.) for 2 hours on the steam-bath gave a crystalline yellow product which, after basification, afforded 1 : 3-dimethyl-2-azafluorenone, m. p. 158—160°, alone or on admixture with an authentic specimen.

Reaction of 9-Bromo-1 : 3-dimethyl-2-azafluorene with Potassium Cyanide.—The bromo-compound (7·35 g.) in ethanol (45 ml.) was treated with potassium cyanide (1·8 g.) dissolved in the minimum quantity of water, and the mixture heated under reflux for 1½ hours. After addition of water and removal of ethanol under reduced pressure, the product was extraced with chloroform, the extract washed with a little dilute acetic acid, then water, and the solvent removed. Crystallisation from light petroleum (b. p. 80—100°) gave a *substance* (3·6 g.), m. p. 202° (Found: C, 83·5; H, 5·8; N, 10·7. $C_{28}H_{23}N_3$ requires C, 83·6; H, 5·7; N, 10·5%). The same product was obtained from 9-chloro-1 : 3-dimethyl-2-azafluorene.

1 : 3-Dimethyl-2-azafluorene.—1 : 3-Dimethyl-2-azafluorenone (10·45 g.), potassium hydroxide (9·5 g.), diethylene glycol (70 ml.), and 100% hydrazine hydrate (5·7 ml.) were heated under reflux at 150° for 1½ hours. The temperature was then raised to 180—190° as rapidly as excessive foaming allowed, and kept there for 4 hours, whereafter evolution of nitrogen had ceased. Next morning the mixture was poured into water (500 ml.), and the precipitated solids were collected and crystallised from light petroleum, to give 1 : 3-dimethyl-2-azafluorene, pale yellow needles (75%), m. p. 93—94° alone or on admixture with an authentic specimen.

Attempted Preparation of 9-Formyl-1 : 3-dimethyl-2-azafluorene.—1 : 3-Dimethyl-2-azafluorene (7·25 g.) was treated with ethyl formate in ether in the presence of potassium methoxide, as described for fluorene by Von and Wagner (*loc. cit.*). The reaction mixture was poured into water and extracted with ether, and the aqueous layer brought to pH 7 by addition of dilute hydrochloric acid. The precipitated gelatinous solid (4·2 g.) was treated with hydroxylamine hydrochloride–sodium acetate in ethanol, giving pale yellow prisms (2·8 g.), m. p. >300°

(Found : C, 51·5; H, 4·0; N, 4·0; Cl, 38·8. 9-Formyl-1 : 3-dimethyl-2-azafluorenone oxime, $C_{15}H_{14}ON_2$, requires C, 75·6; H, 5·9; N, 11·8%). Treatment with alkali furnished a small quantity of 1 : 3-dimethyl-2-azafluorene.

The original product (5·1 g.) was heated with methanol under reflux. The cooled solution deposited yellow needles (1·3 g.), m. p. *ca.* 320° (Found : C, 82·7; H, 6·6; N, 7·4%).

Attempted Preparation of 1 : 3-*Dimethyl*-2-*azafluorene*-9-*carboxylic Acid.*—1 : 3-Dimethylazafluorene (4·88 g.) was condensed with methyl oxalate in the presence of potassium methoxide, as described for fluorene by Campbell and Tucker (*loc. cit.*). The *substance* formed prismatic needles (4·1 g.), m. p. 215° (decomp.) (Found : C, 64·2; H, 5·7; N, 4·7. $C_{16}H_{15}O_5N$ requires C, 63·8; H, 5·0; N, 4·7%). It was insoluble in cold dilute alkali, and, on warming, passed into an oil. Dissolution in dilute hydrochloric acid, followed by basification, led to an oil. Melting was accompanied by rapid evolution of gas. Treatment with picric acid gave yellow needles (from ethanol), m. p. 248—249° (Found : C, 56·8; H, 3·9; N, 12·0%). Pyrolysis in liquid paraffin at 200° gave 1 : 3-dimethyl-2-azafluorene, m. p. 88—90°, alone or on admixture with an authentic specimen.

1 : 3-*Distyryl*-2-*azafluorenone.*—1 : 3-Dimethyl-2-azafluorenone (20·9 g.), benzaldehyde (63·6 g.), and acetic anhydride (61·2 g.) were heated under reflux for 7½ hours. After being kept overnight the separated solids were collected and crystallised from chloroform–light petroleum, to give 1 : 3-*distyryl*-2-*azafluorenone*, bright yellow needles (29·5 g.), m. p. 202° (Found : C, 87·1; H, 5·0; N, 3·7. $C_{28}H_{19}ON$ requires C, 87·3; H, 4·9; N, 3·6%).

2-*Azafluorenone*-1 : 3-*dicarboxylic Acid.*—The foregoing compound (9·6 g.) in pure pyridine (150 ml.) was kept at 55° whilst a warm solution of potassium permanganate (22·5 g.) in water (150 ml.) was slowly added with vigorous stirring during 30—45 minutes. Only gentle external heating was required during the addition. After a further 30 minutes at 55° the precipitated solids were collected and extracted three times with hot water. The bulked filtrate and extracts were evaporated to *ca.* 30 ml. under reduced pressure and then brought to pH 1—2 by addition of concentrated hydrochloric acid. After the mixture had cooled, the precipitated solids were collected, washed free from benzoic acid with water and ether, and crystallised from methanol. 2-*Azafluorenone*-1 : 3-*dicarboxylic acid* (84%) formed yellow needles, m. p. 217° (decomp.) (Found : C, 61·8; H, 3·1; N, 5·3. $C_{14}H_7O_5N$ requires C, 62·4; H, 2·6; N, 5·2%). The *di-silver* salt formed a yellow powder (Found : N, 2·9; Ag, 44·0. $C_{14}H_5O_5NAg_2$ requires N, 3·2; Ag, 44·7%).

2-*Azafluorene.*—Decarboxylation of the foregoing acid by pyrolysis at 20 mm. could only be effected on a 1-g. scale, to give 2-*azafluorenone*, yellow plates (30%) (from aqueous ethanol), m. p. 156—158° (Found : C, 79·5; H, 3·6; N, 7·6. $C_{12}H_7ON$ requires C, 79·6; H, 3·9; N, 7·7%). Reduction gave 2-azafluorene (Mills *et al.*, *loc. cit.*).

1 : 3-*Dimethyl*-9-*nicotinamido*-2-*azafluorene*, feathery needles (from aqueous ethanol), m. p. 271° (Found : C, 72·4; H, 5·6; N, 12·6. $C_{20}H_{17}ON_3,H_2O$ requires C, 72·1; H, 5·7; N, 12·6%), was obtained by treating 9-amino-1 : 3-dimethyl-2-azafluorene (4·2 g.) in pyridine (10 ml.) with nicotinoyl chloride hydrochloride (from 3 g. of nicotinic acid) in pyridine (5 ml.) with external cooling, followed by short heating on a water-bath. The *dihydrochloride* formed feathery crystals (from ethanol–ether), m. p. 325° (Found : C, 61·2; H, 5·0; N, 10·8; Cl, 18·5. $C_{20}H_{17}ON_3,2HCl$ requires C, 61·8; H, 4·9; N, 10·8; Cl, 18·3%).

2-*Diethylaminoethyl* 1 : 3-*dimethyl*-2-*azafluorenone*-4-*carboxylate* separated on cooling a mixture of 1 : 3-dimethyl-2-azafluorenone-4-carboxylic acid (5·0 g.), propan-2-ol (100 ml.), and 2-diethylaminoethyl chloride (3·5 g.) which had been refluxed for 2 hours. It formed crystals, m. p. 58—60°, from water (Found : C, 70·9; H, 6·8; N, 8·2. $C_{21}H_{24}O_3N_2$ requires C, 71·6; H, 6·8; N, 8·0%). The *hydrochloride* formed pale yellow crystals, m. p. 190° (Found : C, 64·9; H, 6·6; N, 7·2; Cl, 9·3. $C_{21}H_{24}O_3N_2,HCl$ requires C, 64·8; H, 6·4; N, 7·2; Cl, 9·1%).

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Chemical Research Laboratories,
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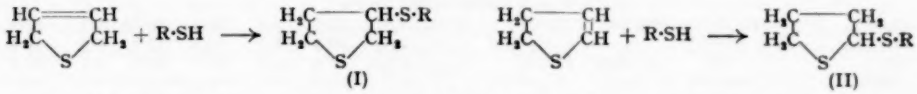
46. *Non-catalytic Reduction of Thiophens. Part III.* The Reaction of the Isomeric Dihydrothiophens with Thiols.*

By S. F. Birch and D. T. McAllan.

The dihydrothiophens react with thiols at 200°, in the presence of elemental sulphur as catalyst, to yield two isomeric series of alkyl (and aryl) tetrahydrothienyl sulphides. These new compounds have been characterised as solid disulphones.

Since olefins react with thiols to form sulphides, the corresponding reaction of dihydrothiophens was investigated with a view to preparing a new type of disulphide containing two non-adjacent sulphur atoms in the molecule.

A great variety of catalysts has been described for the preparation of organic sulphides from olefins and thiols. We adopted Jones and Reid's method (*J. Amer. Chem. Soc.*, 1938, **60**, 2452) in which the reactants were heated in a steel reactor at about 200° for several hours with a trace of elemental sulphur as catalyst. The crude product was an orange-red viscous oil which distilled at reduced pressure to yield a variable amount of unchanged material, a main fraction of high-boiling yellow oil, and a dark polymeric residue.



2 : 5-Dihydrothiophen reacted with ethanethiol, propane-1-thiol, 2-methylpropane-2-thiol, thiophenol, toluene-ω-thiol, *cyclo*hexanethiol, and thioglycollic (mercaptoacetic) acid. The resultant disulphides (I) were characterised by oxidation to the solid disulphones.

2 : 3-Dihydrothiophen reacted similarly with ethanethiol, propane-1-thiol, and toluene-ω-thiol to yield disulphides (II) from which solid disulphones were prepared. The reaction with toluene-ω-thiol was incomplete and the disulphone was isolated as a brown syrup. The reaction with thioglycollic acid produced much hydrogen sulphide and a low yield of crude product which decomposed on distillation.

The addition of thiols to 2 : 5-dihydrothiophen is unambigious and yields only 3-derivatives, but with 2 : 3-dihydrothiophen substitution might occur in the 2- or the 3-position. Since each dihydrothiophen gives different products, it follows that 2 : 3-dihydrothiophen yields only 2-derivatives when treated with thiols in the presence of elemental sulphur. This parallels the work of Kipnis and Ornfelt (*J. Amer. Chem. Soc.*, 1951, **73**, 822) who prepared 2-substituted tetrahydropyranyl sulphides from dihydropyran and thiols in the presence of catalytic quantities of hydrogen chloride.

Experimental

Analyses by Drs. Weiler and Strauss, Oxford.

General Method of Preparation.—The dihydrothiophen (0·25 mol.), slightly less than the theoretical quantity of thiol (0·2 mol.), and elemental sulphur (1 g.) were heated in a steel vessel at 200° (oil-bath) for 8 hours and the resultant red, viscous oil filtered and distilled at reduced pressure. Any unchanged material distilled over first, followed by the main product as a yellow viscous oil; the residue was probably polymeric. When necessary, the sulphide was washed with hot 30% potassium hydroxide solution to remove traces of thiol.

The disulphide (0·05 mol.) was dissolved in acetic acid (60 ml.), and hydrogen peroxide (30 ml.; 100-vol.; 50% excess) added with cooling. The solution was gently heated on the water-bath and, when the initial exothermic reaction had subsided, heating was continued for 3 hours. The solvent was finally removed under reduced pressure and the residual disulphone crystallised to constant m. p.

* Part II, *J.*, 1951, 3411.

The *products* are listed in Tables 1 and 2.

TABLE 1. *Alkyl (or aryl) tetrahydrothienyl sulphides.*

	B. p./mm.	n_D^{20}	Yield, %	Formula	Found, %: C	H	S	Required, %: C	H	S
Tetrahydro-2-thienyl series.										
Et	76—81°/3	1·5500	66	$C_6H_{12}S_2$	49·2	8·1	42·5	48·6	8·2	43·2
Pr^n	85—90/2	1·5410	67	$C_7H_{14}S_2$	52·0	8·8	39·2	51·8	8·7	39·5
Ph	140—145/2·5	1·6250	72	$C_{10}H_{12}S_2$	61·1	6·0	32·9	61·2	6·2	32·7
CH_2Ph	160—165/3	1·605	25	$C_{11}H_{14}S_2$	61·6	6·6	30·6	62·8	6·7	30·5
Tetrahydro-3-thienyl series.										
Et	83/3	1·5485	66	$C_6H_{12}S_2$	49·2	8·4	42·6	48·6	8·2	43·2
Pr^n	95/3·5	1·5350	60	$C_7H_{14}S_2$	51·7	8·8	39·8	51·8	8·7	39·5
Bu^t	90/2	1·5350	34	$C_8H_{16}S_2$	53·3	9·4	36·8	54·5	9·1	36·4
Ph	130—135/1	—	62·5	$C_{10}H_{12}S_2$	61·0	6·4	32·6	61·2	6·2	32·6
CH_2Ph	162/2·5	1·61	56	$C_{11}H_{14}S_2$	62·2	6·7	31·3	62·8	6·7	30·5
*cyclo*Hexyl	144—148/3	1·5580	60	$C_{10}H_{18}S_2$	58·5	8·8	32·0	59·35	9·0	32·0

TABLE 2. *Alkyl (or aryl) sulphonyltetrahydrothiophen 1 : 1-dioxides.*

	M. p.	Crystal form	Solvent *	Formula	Found, %: C	H	S	Required, %: C	H	S
2-Sulphonylthiophen series.										
Et	110° [a]	Plates	EtOH	$C_6H_{12}O_4S_2$	33·8	5·6	30·4	33·95	5·7	30·2
Pr^n	78·5—79·5 [b]	—	C_6H_6–Pet.	$C_7H_{14}O_4S_2$	36·9	6·2	28·5	37·1	6·2	28·4
Ph	164—165	Needles	EtOH	$C_{10}H_{12}O_4S_2$	46·4	4·7	24·7	46·2	4·7	24·6
CH_2Ph	Syrup	—	—	—	—	—	—	—	—	—
3-Sulphonylthiophen series.										
Et	115 [a]	Needles	EtOH	$C_6H_{12}O_4S_2$	34·0	5·8	30·4	33·95	5·7	30·2
Pr^n	88—88·5 [b]	—	C_6H_6	$C_7H_{14}O_4S_2$	37·1	6·6	28·5	37·1	6·2	28·4
Bu^t	172·5—173	Plates	EtOH	$C_8H_{16}O_4S_2$	39·8	6·7	26·8	40·0	6·7	26·7
Ph	161—162	Needles	EtOH	$C_{10}H_{12}O_4S_2$	46·0	4·7	24·8	46·15	4·65	24·6
CH_2Ph	183—184	Needles	$COMe_2$–EtOH	$C_{11}H_{14}O_4S_2$	48·0	5·0	23·3	48·0	5·1	23·3
*cyclo*Hexyl	106—107	—	EtOH	$C_{10}H_{18}O_4S_2$	45·0	6·7	24·1	45·1	6·8	24·1

* Pet. = light petroleum.
[a] Mixed m. p. 85—88°. [b] Mixed m. p. 55—58°.

Reactions with Thioglycollic Acid.—(*a*) 2 : 5-Dihydrothiophen gave a *disulphone* as a benzene-insoluble oil which slowly solidified after several days and crystallised from acetone at low temperature as a brownish solid which decomposed slowly above 150° and liquefied at 210° (Found: C, 28·5; H, 4·8; S, 26·8. $C_8H_{10}O_6S_2$ requires C, 29·8; H, 4·2; S, 26·5%).

(*b*) The reaction of thioglycollic acid and 2 : 3-dihydrothiophen evolved much hydrogen sulphide and distillation of the dark viscous oil gave only a small amount of product up to 150°/3 mm., at which temperature decomposition set in leaving a black tarry residue.

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47. *The Apparent Dielectric Polarisations of (+)- or (−)- and (±)-α-Pinene, -Camphor, -Piperitone, and -Menthol, of Limonene and Dipentene, and of Diethyl Tartrate or Racemate.*

By R. J. W. Le Fèvre and F. Maramba.

The measurements indicated by the title were recorded, with benzene as solvent. As the concentrations of the more polar substances were increased a tendency was noted for the polarisations of (±)-forms to be less than those of the respective (+)- or (−)-optical isomers alone. Some intermolecular interaction dependent upon an antipodal relationship seems therefore to be evident.

The measurements now reported bear upon the possible existence of racemates in the dissolved state; they were made on an assumption that if combination of the (+)- and the (−)-form of a substance were to occur in solution then the apparent polarisation, at a given concentration, of the (±)-mixture would be different from that of either form alone.

By the investigation of the common physical properties, as well as others such as the rate of change of molecular surface energy with temperature (Smith and Mitchell, *J.*, 1913, **103**, 489), magnetic rotatory power (Perkin, *J.*, 1887, **51**, 363; 1902, **81**, 315; 1906, **89**, 849), viscosity (Dunstan, *J.*, 1908, **93**, 1815; 1910, **97**, 1249), or electric double refraction (De Mallemann, *Ann. Physique*, 1924, **2**, 1), earlier investigators seem to have inclined to the belief that the antipodes were not combined in a liquid or molten "racemate" (cf. Findlay, "The Phase Rule," Longmans, Green and Co., 5th edn., 1923, p. 175), although in a number of cases, *e.g.*, molecular refraction (Brühl, *J.*, 1907, **91**, 115), dielectric constant (Stewart, *J.*, 1908, **93**, 1059), and with (+)- and (±)-camphor in De Mallemann's study (*loc. cit.*), there were signs that such a conclusion was not wholly correct, while Fajans's experiments (*Z. physikal. Chem.*, 1910, **73**, 25) on the decomposition rates of (+)- or (±)-camphorcarboxylic acid in acetophenone, like the colour changes described by Cotton (*Ann. Chim. Phys.*, 1896, **8**, 347) on mixing equimolecular solutions of copper (+)- and (−)-tartrates, gave strong indications of mutual interaction or an equilibrium: $(\pm) \rightleftharpoons (+) + (-)$.

The general question has been considered more recently by Campbell (*J.*, 1929, 1111) and Cotton (*Trans. Faraday Soc.*, 1930, **26**, 377). The latter quotes facts to show that, in water, salts of racemic acid are different from equivalent mixtures of the corresponding two tartrates, while the former comments on the "very conflicting nature of the evidence and of opinions" in the literature, and presents evidence that the viscosities of ethyl, propyl, butyl, and amyl tartrates as pure liquids exceed those of the related racemates by amounts well beyond observational errors. For the ethyl esters, included in the present work, the ratio $\eta_{\text{tartrate}}/\eta_{\text{racemate}}$ was 1·51 at 18°.

The first relevant dipole-moment studies on benzene solutions were made by Weissberger and Sängewald (*Z. physikal. Chem.*, 1931, *B*, **13**, 383) who examined the methyl esters of mandelic, tartaric, and racemic acids; within the accuracy of their published measurements the moments of the (+)- and the (±)-forms appeared not to differ by more than 0·2 D. Coppock and Goss (*J.*, 1939, 1789) later found the partial polarisations of (+)-, (−)-, or (±)-octan-2-ols in benzene to be identical to the nearest c.c. over a composition range from 0 to 1 molar fraction. They concluded therefore that the inactive alcohol is simply a racemic mixture.

After this work was started, we found that Sahney, Barucha, and Sarna (*J. Indian Chem. Soc.*, 1948, **25**, 285) had made similar measurements on, *inter alia*, the camphors. We refer to their results later.

In this paper we record density and dielectric-constant observations at 25° for mixtures with benzene of the substances named in the title. Instead of directly estimating the solute polarisations by mixture formulæ, we have proceeded as set out by Le Fèvre (*Trans.*

Faraday Soc., 1950, **46**, 1) and fitted experimental values of $(\varepsilon_{12} - \varepsilon_2)/w_1$ and $(d_{12} - d_2)/w_1$ —written as $(\alpha\varepsilon_2)_{w_1}$ and $(\beta d_2)_{w_1}$ respectively in what follows—to equations of the type $(\alpha\varepsilon_2)_{w_1} = A + Bw_1$ (cf. Mellor, "Higher Mathematics," Longmans, Green and Co., 1931, p. 326).

Experimental.

Materials and Measurements.—Commercial specimens (from B.D.H., London) of the two active pinenes were steam-distilled, separated from water, and roughly fractionated without drying. The portions boiling at 155·5—156·5° were partially frozen (as described for *p*-cymene in *J.*, 1933, 980), the solid obtained after draining being then melted, dried (Na_2SO_4), and redistilled. The main bulk, boiling at 156°/760 mm. in each case, was stored over sodium for use in this work. The inactive material was made by mixing the (+)- with a slight excess of the (−)-form until the polarimetric reading was *ca.* 0°. Rotations, densities, and dielectric constants recorded were:

	$[\alpha]_D^{19}$	d_4^{25}	ε^{25}	n_D^{15}
(+)-α-Pinene	+ 44·2°	0·8540	2·3684	1·4652
(−)- ,, ...	− 39·4	0·8546	2·3701	1·4655
(±)- ,, ...	*ca.* 0	0·8547	2·3695	1·4645

Stewart (*loc. cit.*) gives ε^{20} of (+)-, (−)-, and (±)-pinene as 2·60, 2·70, and 2·75, Müller (*Physikal. Z.*, 1937, **38**, 283) those of (−)- and (±)-pinene as 2·762 and 2·42, respectively, and Svirbely, Ablard, and Warner (*J. Amer. Chem. Soc.*) give ε^{25} of (+)-pinene as 2·6428. Our measurements are lower than these; they correspond to a total polarisation (50·0 c.c.) which is about 1·5 c.c. less than that at infinite dilution in benzene (see below). However, part of the above (±)-pinene stock, after it had been in an ordinary stoppered bottle (*i.e.*, in partial contact with atmospheric moisture, air, and light) for about two months, was found to have ε^{25} *ca.* 2·6. The higher figures reported by earlier authors may therefore have been due to the unsuspected presence of polar moloxides of pinene. A note by Walker (*J.*, 1925, **127**, 1491) suggests that some such addition products with oxygen are formed very readily.

Limonene was purchased (B.D.H., London), the dipentene prepared from α-terpineol (Wall, *Annalen*, 1893, **275**, 104). Both were purified as described for the pinenes, and finally collected at 177—178°/760 mm. Properties recorded were:

	$[\alpha]_D^{18}$	d_4^{25}	ε^{25}	n_D^{25}
(+)-Limonene.....	+ 98°	0·8423	2·446	1·4759
(±)-Dipentene	0	0·8445	2·452	1·4785

Stewart (*loc. cit.*) found ε^{20} to be 2·36 and 2·30, respectively. On our figures the total polarisations are 52·3 and 52·6 c.c.

A sample of natural "refined camphor," obtained from the Taiwan Camphor Bureau, Taipeh, Taiwan, China, and purified by sublimation, had $[\alpha]_D^{20}$ +44·7° (10% alcoholic solution). Inactive camphor was synthetic material from the Eastman Kodak Co. It was also purified by sublimation.

Kannonikow (*J. pr. Chem.*, 1885, **32**, 504) reported the $[R_L]_a$ for camphor as 44·4 c.c. Using sodium light, we found an 8·722% solution in benzene (d_D^{25} 0·87378, n_D^{25} 1·4972) to have d_D^{25} 0·88033 and n_D^{25} 1·4942, whence the molecular refraction of the solute is 43·7 c.c. Since only an Abbé refractometer was available, in the following tables we have used the value (44·0 c.c.) calculated from Vogel's constants (*J.*, 1948, 1842).

A crude sample of (−)-piperitone extracted from the oil of *Eucalyptus dives* was fractionated and a portion having b. p. 93·0°/5 mm., $[\alpha]_D^{20}$ −38·5°, n_D^{20} 1·4848, selected for the present work. An impure specimen of the above piperitone was well shaken with a saturated solution of sodium hydrogen sulphite. The resulting complex was washed with alcohol and with ether, then dissolved in hot water, filtered, and made alkaline, and the regenerated ketone separated. After being dried (Na_2SO_4), a fraction of b. p. 117°/18·5 mm., n_D^{20} 1·4845, was collected. On a second occasion these operations were repeated with a new supply of piperitone; the product, however, although showing the same b. p. and n_D^{20}, seemed to be slightly more polar than the first. Six solutions of this sample are reported in the following tables—they may be distinguished by $\alpha\varepsilon_2$ values of 10·0 or higher.

The menthols used were commercial samples; recrystallisation of the synthetic (±)-material from light petroleum gave needles, m. p. 34·5°, which were not quite optically inactive ($[\alpha]_D^{16}$

−1·85°); the natural (−)-menthol was redistilled, b. p. 104—105°/10 mm., and used without further purification (cf. Pickard and Littlebury, *J.*, 1912, **101**, 109).

Ethyl tartrate (from B.D.H., London) was refractionated before use, b. p. 142°/8 mm., $[\alpha]_D^{16}$ 7·9°. Ethyl racemate proved somewhat difficult to prepare. The acid (15 g.; cf. *Org. Synth.*, **6**, 88) was dehydrated at 110° for 6 hours, then finely powdered, and absolute alcohol (18 c.c.) added. The mixture was saturated with dry hydrogen chloride, first on the water-

10^5w_1	ε^{25}	$a\varepsilon_2$	d_4^{25}	βd_2		10^5w_1	ε^{25}	$a\varepsilon_2$	d_4^{25}	βd_2	
0	2·2725	—	0·87378	—		—	—	—	—	—	
					α-Pinenes.						
590·2	2·2733	0·136	0·87366	−0·020	(+) *	703·1	2·2736	0·156	0·87365	−0·018	(±)
609·1	2·2735	0·164	0·87365	−0·021	(−)	1015·8	2·2743	0·177	0·87358	−0·020	(±)
1050·8	2·2743	0·171	0·87358	−0·019	(−)	1179·1	2·2746	0·178	0·87358	−0·017	(±)
1657·0	2·2757	0·193	0·87350	−0·017	(+)	4099·0	2·2803	0·188	0·87299	−0·019	(±)
5237·0	2·2830	0·200	0·87283	−0·018	(−)	5011·8	2·2825	0·200	0·87284	−0·019	(±)
5584·0	2·2834	0·195	0·87276	−0·018	(+)						
whence $(a\varepsilon_2)_{w_1} = 0·157 + 0·8w_1$, and mean $\beta d_2 = -0·018_8$.						whence $(a\varepsilon_2)_{w_1} = 0·163 + 0·7w_1$, and mean $\beta d_2 = -0·018_6$.					
					Limonene.						*Dipentene.*
905·1	2·2745	0·221	0·87348	−0·033	(+)	793·8	2·2743	0·227	0·87354	−0·030	(±)
1099·5	2·2751	0·236	0·87344	−0·031	(+)	995·2	2·2748	0·231	0·87346	−0·032	(±)
2173·1	2·2774	0·225	0·87308	−0·032	(+)	1867·1	2·2768	0·230	0·87322	−0·030	(±)
2211·6	2·2775	0·226	0·87308	−0·032	(+)	2089·6	2·2772	0·225	0·87316	−0·030	(±)
whence mean $a\varepsilon_2 = 0·227$, and mean $\beta d_2 = -0·032$.						whence mean $a\varepsilon_2 = 0·228$, and mean $\beta d_2 = -0·030_5$.					
					Camphors.						
545	2·3101	6·90	0·87443	0·119	(+)	509	2·3081	7·00	0·87436	0·114	(±)
955	2·3379	6·85	0·87488	0·115	(+)	1077	2·3456	6·79	0·87493	0·107	(±)
1484	2·3734	6·80	0·87513	0·091	(+)	1443	2·3688	6·68	0·87510	0·092	(±)
1850	2·3952	6·63	0·87527	0·081	(+)	4546	2·5636	6·70	0·87776	0·092	(±)
5283	2·6258	6·69	0·87812	0·083	(+)	9306	2·8929	6·67	0·88150	0·083	(±)
8685	2·8587	6·75	0·88101	0·083	(+)						
whence $(a\varepsilon_2)_{w_1} = 6·81 - 1·3w_1$, and $(\beta d_2)_{w_1} = 0·106 - 0·34w_1$.						whence $(a\varepsilon_2)_{w_1} = 6·84 - 2·2w_1$, and $(\beta d_2)_{w_1} = 0·107 - 0·28w_1$.					
					Piperitones.						
143·1	2·2867	9·92	0·87385	0·049	(−)	362·8	2·3088	10·00	0·87394	0·044	(±)
393·3	2·3112	9·84	0·87392	0·036	(−)	391·7	2·3109	9·80	—	—	(±)
649·4	2·3350	9·63	0·87401	0·035	(−)	428·4	2·3150	9·92	0·87396	0·042	(±)
892·4	2·3586	9·65	0·87409	0·035	(−)	545·9	2·3289	10·33	—	—	(±)
1035·5	2·3725	9·66	0·87417	0·038	(−)	896·0	2·3640	10·21	0·87411	0·037	(±)
3169·7	2·5709	9·41	0·87519	0·044	(−)	1311·4	2·4081	10·34	—	—	(±)
whence $(a\varepsilon_2)_{w_1} = 9·84 - 14·7w_1$, and mean $\beta d_2 = 0·039_5$.						1555·8	2·4277	9·97	0·87455	0·043	(±)
						2030·4	2·4823	10·33	—	—	(±)
						2677·4	2·5503	10·37	0·87494	0·043	(±)
						whence mean $a\varepsilon_2 = 10·14$, and mean $\beta d_2 = 0·042$.					
					Menthols.						
119·1	2·2750	2·10	0·87381	0·025	(−)	153·4	2·2758	2·15	0·87385	0·046	(±)
404·7	2·2804	1·95	0·87387	0·032	(−)	353·0	2·2796	2·01	0·87394	0·045	(±)
536·6	2·2837	2·09	0·87395	0·032	(−)	859·1	2·2882	1·83	0·87433	0·064	(±)
898·2	2·2896	1·90	0·87398	0·022	(−)	989·0	2·2930	2·07	0·87418	0·040	(±)
1757·5	2·3048	1·84	0·87468	0·051	(−)	1717·0	2·3048	1·88	0·87536	0·092	(±)
7568·7	2·4113	1·83	0·87500	0·016	(−)	3873·8	2·3453	1·88	0·87448	0·028	(±)
whence $(a\varepsilon_2)_{w_1} = 2·00 - 2·61w_1$, and $(\beta d_2)_{w_1} = 0·033 - 0·18w_1$.						whence $(a\varepsilon_2)_{w_1} = 2·04 - 5·2w_1$, and $(\beta d_2)_{w_1} = 0·057 - 0·36w_1$.					
					Ethyl tartrate.						*Ethyl racemate.*
220·8	2·2863	6·25	0·87430	0·236	(+)	255·5	2·2888	6·38	0·87435	0·262	(±)
603·1	2·3088	6·02	0·87522	0·239	(+)	598·3	2·3089	6·08	0·87506	0·214	(±)
972·1	2·3318	6·10	0·87629	0·258	(+)	668·3	2·3102	5·79	0·87508	0·195	(±)
1121·3	2·3388	5·91	0·87679	0·268	(+)	1102·2	2·3348	5·65	0·87679	0·273	(±)
1669·1	2·3705	5·87	0·87827	0·269	(+)	2389·4	2·4041	5·51	0·88092	0·304	(±)
4743·9	2·5304	5·44	0·88761	0·296	(+)	3586·8	2·4893	5·53	0·88164	0·219	(±)
whence $(a\varepsilon_2)_{w_1} = 6·18 - 16·2w_1$, and $(\beta d_2)_{w_1} = 0·242 + 1·23w_1$.						whence $(a\varepsilon_2)_{w_1} = 6·12 - 20·6w_1$, and $(\beta d_2)_{w_1} = 0·238 + 0·419w_1$.					

* This column shows the optical form of the solute.

bath and finally under ice-cooling. After 24 hours the whole was roughly distilled under reduced pressure. Such ester as had formed was then returned, plus more absolute alcohol, to the reaction flask, and the hydrogen chloride saturation repeated until all the racemic acid had disappeared. Finally, distillation, extraction with ether, neutralisation with aqueous sodium carbonate, drying (Na_2SO_4), etc., afforded a preparation with b. p. 138°/5 mm. (Beilstein, " Handbuch," III, 527, quotes b. p. 157°/11·5 mm.).

The measurements tabulated above have been obtained by means of the apparatus and methods described before (cf. *J.*, 1948, 1949; 1949, 333; " Dipole Moments," Methuen, 1948, Chapter II). The symbol convention follows that used in *Trans. Faraday Soc.*, 1950, **46**, 1, wherein extrapolation procedures are also discussed. The solvent has been benzene throughout and all d and ε determinations have been fixed relatively to the d_2 and ε_2 values shown for $w_1 = 0$.

From the above and other data the apparent dipole moments of the solutes are as shown in the last column of the following table:

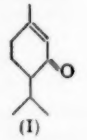
	M_1	$(a\varepsilon_2)_{w_1=0}$	$(\beta_{w_1})_{=0}$	$_\infty P_1$ (c.c.).	$[R_L]_D$, c.c.	μ, D
(+)- or (−)-α-Pinene...	136·2	0·157	$-0·021_5$	51·4	44·1	$0·6_0$
(±)-α-Pinene	,,	0·163	$-0·021_3$	51·6	44·0	$0·6_1$
(+)-Limonene	,,	0·227	$-0·036_8$	53·9	45·6	$0·6_4$
(±)-Dipentene	,,	0·228	$-0·034_9$	53·9	45·7	$0·6_3$
(+)-Camphor	152·2	6·81	0·121	240·6	44·0	$3·1_0$
(±)-Camphor	,,	6·84	0·122	241·4	44·0	$3·1_0$
(−)-Piperitone	,,	9·84	0·045	331·2	46·8 [1]	$3·7_3$
(±)-Piperitone	,,	10·14	0·048	339·7	46·7 [1]	$3·7_8$
(−)-Menthol	156·2	2·00	0·038	110·0	47·6 [2]	$1·7_5$
(±)-Menthol	,,	2·04	0·065	109·7	47·7 [3]	$1·7_4$
Ethyl tartrate	206·2	6·18	0·227	290·5	45·6 [4]	$3·4_6$
Ethyl racemate	,,	6·12	0·272	288·5	45·5 [5]	$3·4_5$

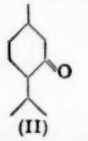
[1] Read and Smith, *J.*, 1923, **123**, 2268. [2] Quoted by Guenther, " The Essential Oils," Van Nostrand Co., 1949, Vol. II, p. 255. [3] Zeitschel and Schmidt, *Ber.*, 1926, **59**, 2298. [4] Peacock, *J.*, 1915, **107**, 1553. [5] Kuhn and Wagner-Jauregg, *Ber.*, 1928, **61**, 491.

Discussion

(a) *The Apparent Dipole Moments.*—Svirbely, Ablard, and Warner (*J. Amer. Chem. Soc.*, 1935, **57**, 652) reported moments for (+)-pinene and (+)-limonene, in benzene, of 2·67 and 1·56 D, respectively. Otto (*ibid.*, p. 1147), by recalculations from the experimental data, amended the final results to the—still high—figures 2·60 and 1·57 D. Müller (*Physikal. Z.*, 1937, **38**, 283), however, found for (±)- and (−)-pinene polarities, 0·6 and 0·8 D, more in line with other olefinic hydrocarbons. Later work by Davis, Bridge, and Svirbely (*J. Amer. Chem. Soc.*, 1943, **65**, 857) afforded for pinene in benzene a total polarisation at infinite dilution at 30·1° of 49·0 c.c.; we now find 51—52 c.c. at 25°. For limonene or dipentene we can find no other measurements in the literature since the 1935 reference above. As comparisons the following moments may be cited:

	μ, D	
$Me_2C{:}CH_2$	0·37	Smyth and Zahn (*J. Amer. Chem. Soc.*, 1925, **47**, 2501).
$MeCH{:}CH_2$	0·35	McAlpine and Smyth (*ibid.*, 1933, **55**, 453).
Various methyl- and dimethyl-butadienes	Up to 0·59	Farmer and Warren (*J.*, 1933, 1297, 1302).
*cyclo*Hexene	0·61	Kubo (*Sci. Papers, Inst. Phys. Chem. Res. Tokyo*, 1937, **31**, 296).


(I)


(II)

(III)

(IV)

The present appear to be the first determinations on piperitone (I), whose moment, 3·7—3·8 D, is in accordance with expectations for a molecule containing the conjugated unit $C{=}C{-}C{=}O$ Thus we note that the difference between (I) and its dihydro-derivative, menthone (II; $\mu = ca.$ 2·8 D, cf. M.I.T. Tables), is equal to that between *cyclo*hexanone (III; $\mu = 3·0$ D) and 3 : 5-dimethyl*cyclo*hex-2-enone (IV; $\mu = 4·0$ D; Bentley, Everard, Marsden, and Sutton, *J.*, 1949, 2957).

With the camphors, menthols, and ethyl racemate, the moments derived from our observations are somewhat higher than those given by previous workers :

Camphor : $\mu = 2{\cdot}95$ D (Donle and Volkert, *Z. physikal. Chem.*, 1930, *B*, **8**, 60); 2·94 (Wolf, *Physikal. Z.*, 1930, **31**, 227); 3·05 (Higasi, *Bull. Inst. Phys. Chem. Res. Tokyo*, 1932, **11**, 729); 2·90—2·91 (Sahney, Barucha, and Sarna, *loc. cit.*); 3·10 (present work).

Menthol : $\mu = 1{\cdot}58$ D (Donle and Wolf, *Z. physikal. Chem.*, 1930, *B*, **8**, 55); 1·62 (Harms, *ibid.*, 1934, *B*, **30**, 440); 1·54—1·55 (Sahney *et al.*, *loc. cit.*); 1·74—1·75 (present work).

Ethyl racemate : $\mu = 3{\cdot}1$—$3{\cdot}2$ D (Wolf, *Trans. Faraday Soc.*, 1930, **26**, 315; Wolf and Bodenheimer, *Z. physikal. Chem.*, Bodenstein Festschr., 1931, p. 620); 3·45 (present work).

Inspection of the actual concentration, dielectric constant, and density figures (when given) presented in the above papers, suggests that the disagreements have an experimental origin, and are neither due to the mathematical extrapolative procedure used by us—although this can sometimes lead to higher estimates of ${}_{\infty}P_1$ than would the older graphical method (cf. Le Fèvre and Russell, *J.*, 1936, 491, for examples)—nor to different allowances for the distortion polarisations.

(b) *The Concentration–Polarisation Relations for Active and Inactive Forms.*—In an optically inactive solvent the total molecular polarisation at infinite dilution of the (+)- and the (−)-form of a solute should be identical. If therefore a (±)-solute were merely a mixture, the constants given by the equations for $(\alpha\varepsilon_2)_{w_1}$ versus w_1—or $(\beta d_2)_{w_1}$ versus w_1—should, when $w_1 = 0$, be the same as for one of the antipodes alone. Among our results this is seen nearly to be realised with limonene and dipentene and with (+)- and (±)-camphor, and to lesser degrees with the other substances. [We except the piperitones since the (±)-form seemed to contain an unknown polar impurity, cf. above.] The differences are not greater than the irregularities among the individual observations for each form shown in the tables. We conclude therefore that our measurements afford no evidence against the view that the (+)- and the (−)-component of a dissolved (±)-preparation exist independently of one another at high dilutions.

The coefficients of w_1 in the equations for $(\alpha\varepsilon_2)_{w_1}$ and $(\beta d_2)_{w_1}$ suggest that in the cases of the pinenes, and of limonene and dipentene, this independence continues throughout the short concentration ranges tabulated (*ca.* 0—5% and 0—2%). For the remaining substances, however, plots of p_1 [evaluated *via* $p_1 = p_2 + (p_{12} - p_2)/w_1$; cf. Sugden, *Trans. Faraday Soc.*, 1934, **30**, 720] versus w_1 are not the same for each optically active and inactive solute pair. The differing effects of concentration can be illustrated by calculating ε_{12} and d_{12} for $w_1 = 0{\cdot}05$ and 0·10, using the equations for $(\alpha\varepsilon_2)_{w_1}$ and $(\beta d_2)_{w_1}$, and therefrom—by the mixture rule—the apparent values of P_1 at these common concentrations :

Camphors.			Menthols.			Ethyl tartrate and racemate.		
(+)	224·7	211·7	(−)	105·5	104·0	(+)	238·9	195·5
(±)	224·0	209·8	(±)	102·0	95·0	(±)	235·4	187·8

It thus seems that in more concentrated solutions the apparent polarisation of a (±)-form becomes relatively less than that of either the (+)- or (−)-form alone : alternatively expressed, our measurements indicate some slight mutual interaction between the dissolved antipodes. Sahney, Barucha, and Sarna (*loc. cit.*) reached a similar conclusion, quoting for 10% weight/volume concentrations in benzene at 20° the following excesses of $(P_1)_{\text{active}}$ over $(P_1)_{\text{inactive}}$: Camphor, 2; borneol, 2; menthol, 3; camphorquinone, 8 c.c.

These results are explicable if association occurs more easily between antipodal molecules than between those of one kind, and if the (±)-complex so produced is more nearly centrosymmetric than is each active component alone. From the mirror-image relationship of the species concerned, the second supposition would seem to be reasonable. As to the forces holding the (+)- and (−)-units together, dipole–dipole and dipole–induced dipole attractions are an obvious guess; we note that solid (+)- or (−)-tartrates have recently been reported to show infra-red spectra different from those of racemates (Duval and Lecomte, *J. Chem. Physics*, 1950, **18**, 1117; cf. Turner and Lonsdale, *ibid.*, p. 156), indicating that in the latter the (+)- and (−)-anions cannot merely be in juxtaposition

but must also be influencing one another's link moments, polarisabilities, force constants, etc. Again, whenever, as with camphor and menthol, the inactive form differs from the active in m. p. and other crystal properties (*e.g.*, dielectric relaxation; cf. Kauzmann, *Rev. Mod. Physics*, 1942, **14**, 40) it is obvious that the components undergo some modification in their union. If in such instances we regard the solid phase as the limiting case among progressively more concentrated solutions, then the findings now reported from our experiments are not unexpected.

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48. *The Q-Enzyme of* Polytomella coeca.*

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For the synthesis of starch, *Polytomella coeca* utilises a phosphorylase and a Q-enzyme. The latter has been purified and shown to be very similar to potato Q-enzyme; it converts amylose into a polysaccharide which stains reddish-purple with iodine, there being little liberation of reducing sugar. The course of this conversion has been examined in detail. The new Q-enzyme and potato phosphorylase together show an autocatalytic liberation of inorganic phosphate when incubated with glucose-1 phosphate; the polysaccharide produced stains reddish-purple with iodine.

Polytomella coeca is a flagellated protozoa belonging to a group of organisms which are of particular interest inasmuch as they do not contain chlorophyll and yet are able to synthesise relatively large quantities of starch from simple carbon sources, but not from sugars (Lwoff, Ionesco, and Gutmann, *Biochim. Biophys. Acta*, 1950, **4**, 270). This organism has been extensively studied by Lwoff (*Ann. Inst. Pasteur*, 1941, **66**, 407) and Lwoff, Ionesco, and Gutmann (*loc. cit.*; *Compt. rend.*, 1949, **228**, 342), who have shown that it grows readily between pH 3·0 and 8·0 in a purely synthetic medium containing ammonium sulphate as the nitrogen source and acetic acid or ethyl alcohol as the carbon source; in addition, inorganic phosphate and traces of iron, magnesium, and thiamine are necessary. A culture medium such as this, in which radioactive sodium acetate is incorporated, furnishes a fruitful source of labelled starch (Bevington, Bourne, and Wilkinson, *Chem. Ind.*, 1950, 691).

In two recent papers Lwoff, Ionesco, and Gutmann (*locc. cit.*) reported the isolation from *Polytomella coeca* of an enzyme preparation containing phosphorylase and showed that the immediate precursor of the starch formed by the organism was glucose-1 phosphate. In the present work we have confirmed that a cell-free extract of the flagellate is capable of synthesising an iodine-staining starch-type polysaccharide from the Cori ester, but not, under the same conditions, from glucose, maltose, or sucrose. Thus it is very probable that *Polytomella coeca* synthesises chains of 1 : 4-α-linked glucose units by the agency of a phosphorylase, rather than of an amylosucrase (cf. Hehre and Hamilton, *J. Bact.*, 1948, **55**, 197; Hehre, Hamilton, and Carlson, *J. Biol. Chem.*, 1949, **177**, 267) or an amylomaltase (Monod and Torriani, *Compt. rend.*, 1948, **227**, 240; 1949, **228**, 718; Doudoroff *et al.*, *J. Biol. Chem.*, 1949, **179**, 921).

In a detailed examination of the composition of the starch formed by the intact cells of *Polytomella coeca*, three of us (*J.*, 1950, 2694) found that the major component, representing 84—87%, possessed the ramified amylopectin structure. Now, it is well known that phosphorylases isolated from plant and animal sources synthesise only amylose and that a second enzyme is necessary to establish the branch points found in amylopectin. This supplementary enzyme, which has been termed Q-enzyme, was first isolated by Haworth, Peat, and Bourne (*Nature*, 1944, **154**, 236) from potatoes and was obtained in crystalline form from that source by Gilbert and Patrick (*Nature*, 1950, **165**, 573). It is

* Presented, in brief, at the XIIth International Congress of Pure and Applied Chemistry, New York September 1951.

known to occur also in the broad bean and the wrinkled pea (Hobson, Whelan, and Peat, *J.*, 1950, 3566). Q-Enzyme appears to be closely related to the branching factor which is involved in the synthesis of glycogen (cf. Cori and Cori, *J. Biol. Chem.*, 1943, **151**, 57; Hehre, *ibid.*, 1949, **177**, 267). We now report the isolation of a Q-enzyme from *Polytomella coeca*.

In the standard method for the preparation of the enzyme the bacterial cells were ruptured by grinding them with sand, and an aqueous extract of the debris was treated with lead acetate in a manner similar to that used by Barker, Bourne, and Peat (*J.*, 1949, 1705) in the case of the potato enzyme. The precipitated lead–protein complex was eluted with sodium hydrogen carbonate solution in a stream of carbon dioxide and the Q-enzyme was precipitated from the extract with neutral ammonium sulphate, at a concentration (25 g./100 c.c.) chosen to give maximal activity consistent with the removal of phosphorylase. No amylose-precipitating fatty acid was detected in any sample of Q-enzyme thus prepared. The activity of the enzyme was appreciably greater than that of an equal quantity of potato Q-enzyme at the same stage of purification. An alternative

FIG. 1. *Action of Q-enzyme on amylose.*

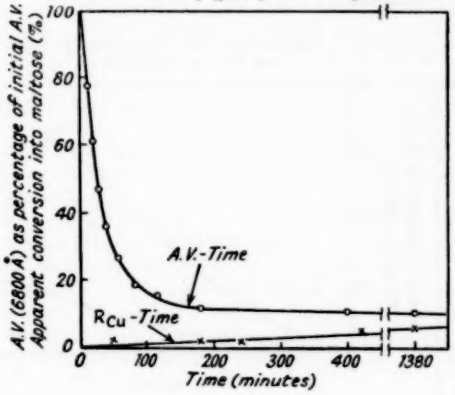
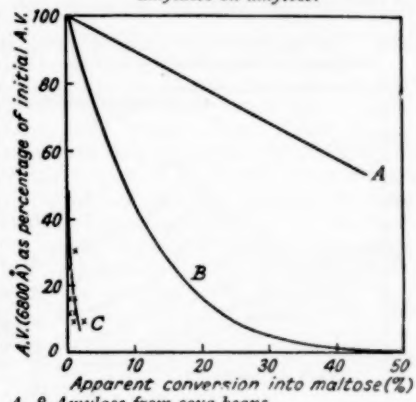


FIG. 2. *The action of Q-enzyme and amylases on amylose.*



A, β-Amylase from soya beans.
B, Salivary α-amylase.
C, Composite curve of results with potato Q-enzyme and Polytomella coeca Q-enzyme.

process, in which the lead–protein complex was eluted with citrate buffer (pH 7·0) instead of sodium hydrogen carbonate solution, was not adopted because, although it gave a slightly better yield of Q-enzyme activity, there was a greater contamination with both amylase and non-enzymic protein impurities. However, there is one respect in which the citrate elution process offers an advantage, namely, that the Q-enzyme sample finally obtained from such a process can be freeze-dried in 0·2M-citrate buffer (pH 7·0) with negligible loss in activity, whereas under similar drying conditions the product of the standard sodium hydrogen carbonate process loses almost the whole of its Q-enzyme activity. Thus when the Q-enzyme is to be stored over a prolonged period it is better that it should be kept as the less pure freeze-dried product of the citrate elution process and then purified further when required. It seems probable that the extraneous proteins eluted in the citrate method stabilise the Q-enzyme during drying (cf. Barker, Bourne, and Peat, *loc. cit.*; Barker, Bourne, Wilkinson, and Peat, *J.*, 1950, 84); the prevention of the development of acidity during freeze-drying is not in itself sufficient protection against deactivation of the enzyme.

Samples of Q-enzyme obtained from the flagellate by the standard method rapidly converted potato amylose into a polysaccharide which stained reddish-purple with iodine. The changes in the iodine-staining properties and reducing power of a typical digest of this type are shown in Fig. 1. It will be seen that the A.V. (6800 Å) of the solution decreased

rapidly, falling to 11% of its initial value in 3 hours; thereafter there was a further decrease of only 2% during the next 20 hours. Since the "apparent conversion into maltose," as measured by cuprimetric titration, was only 2 and 6% after 3 and 23 hours, respectively, there could have been very little, if any, contamination with either α- or β-amylase. When the time factor was eliminated by plotting A.V. (6800 Å) against "apparent conversion into maltose" the curve given in Fig. 2 was obtained; the points shown in this figure represent the results of several experiments in which different samples of Q-enzyme were employed. The striking similarity between this enzyme and potato Q-enzyme and their non-identity with either α- or β-amylase is clearly demonstrated. These results were consistent with the conversion of amylose into an amylopectin-type product by the Q-enzyme of *Polytomella coeca*, as also were the changes in the complete light-absorption curves of the polysaccharide–iodine solutions (Fig. 3).

The optimum pH for the amylose conversion is *ca.* 7·3, compared with 7·0 for potato Q-enzyme (Barker, Bourne, and Peat, *loc. cit.*) and 7·25—7·5 for the Q-enzyme of the broad bean (Hobson, Whelan, and Peat, *loc. cit.*). The optimum temperature is particularly sensitive to pH change and values ranging from 25° to 33° have been obtained; Barker, Bourne, and Peat (*loc. cit.*) recorded 21° ± 1° for potato Q-enzyme, and Hobson, Whelan, and Peat (*loc. cit.*) gave 20° for broad bean Q-enzyme.

FIG. 3. *Light-absorption curves obtained during the treatment of amylose with Q-enzyme.*

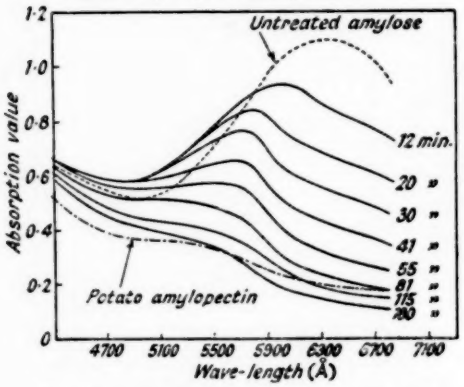
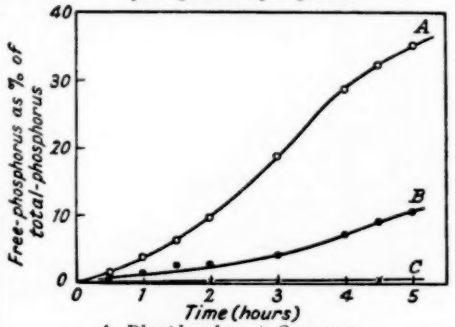


FIG. 4. *Autocatalysis in polysaccharide synthesis from glucose-1 phosphate.*



A, Phosphorylase + Q-enzyme.
B, Phosphorylase alone.
C, Q-Enzyme alone.

The Q-enzyme of *Polytomella coeca* had, *per se*, no action on glucose-1 phosphate, and therefore was free from phosphorylase. However, like potato Q-enzyme (Barker, Bourne, Wilkinson, and Peat, *loc. cit.*) and the "branching factor" of liver and heart (Cori and Cori, *loc. cit.*), it promoted the synthetic function of potato phosphorylase; this autocatalytic effect can be seen in Fig. 4. The joint action of the two enzymes resulted in the formation of a polysaccharide which stained reddish-purple with iodine. Although this effect is not specific for Q-enzyme, it does reveal the presence of an enzyme which increases the number of non-reducing chain-ends in the amylose produced by the phosphorylase action.

In conclusion, an enzyme similar to potato Q-enzyme has been isolated from *Polytomella coeca*; this enzyme catalyses the conversion of amylose into a polysaccharide which stains reddish-purple with iodine and may well be of the amylopectin-type. The amylose conversion has now been carried out on a relatively large scale and the polysaccharide product has been isolated; as will be shown in the following paper, rigorous chemical examinations have revealed that it is a member of the amylopectin–glycogen class.

EXPERIMENTAL

Definitions of Starch–Iodine Colours.—Two expressions, absorption value (A.V.) and blue value (B.V.), are used in this paper to describe the intensities of the iodine stains of polysaccharides; they were defined by Bourne, Haworth, Macey, and Peat (*J.*, 1948, 924). Since the B.V. is determined under standard conditions (polysaccharide, 1 mg./100 c.c.; iodine,

2 mg./100 c.c.; potassium iodide, 20 mg./100 c.c.) and at λ 6800 Å, it is characteristic of the polysaccharide under examination. On the other hand, the A.V. is a convenient term for an absorption measurement made under any given set of conditions, for example, during the course of a reaction in which the polysaccharide concentration may be changing.

Storage and Culture of Polytomella coeca (cf. Lwoff, Ionesco, and Gutmann, *loc. cit.*).—(a) *In a peptone medium.* For maintaining the culture over long periods a 0·5% peptone broth, adjusted with sodium hydroxide to pH 6·0, was employed. The medium was sterilised for 30 minutes at 15 lb. pressure. When it was cool, it was inoculated with a pure culture of *Polytomella coeca* and kept in the dark at room temperature. When a mass culture was required, ethyl alcohol (1 c.c. per 250 c.c. of culture) was added and in 2—3 days vigorous growth occurred.

(b) *In a synthetic medium.* The synthetic medium consisted of ammonium sulphate (4·0 g.), magnesium sulphate (0·4 g.), sodium acetate (4·0 g.), potassium dihydrogen phosphate (2·0 g.), ethyl alcohol (12·0 c.c.), and thiamine hydrochloride (0·04 mg.) in distilled water (4 l.). It was adjusted to pH 7·0 with sodium hydroxide and sterilised for 40 minutes at 15 lb. pressure. When it was cool, 0·4% ferric ammonium citrate solution (10 c.c.), which had been sterilised separately, was added.

In order to obtain a mass culture, a portion (250 c.c.) of the synthetic medium was inoculated with a portion (2 c.c.) of a young mass peptone culture, and incubated at 25°, whilst being continuously aerated with a fine stream of air, which had been previously freed from carbon dioxide. After 2—3 days, a portion (25 c.c.) of the vigorous culture was introduced into a large volume (4 l.) of the synthetic medium, which was then incubated for 3—5 days, as before.

Standard Method for the Isolation of Q-Enzyme.—A mass culture was grown in a synthetic medium (4 l.), as described above. The flagellates were collected in the centrifuge and ground with washed white sand until microscopical examination revealed that almost complete disruption of the cells had been effected. The ground mass was extracted with three portions (15 c.c. each) of 0·02M-citrate buffer (pH 7·0), and the volume of the combined extracts was adjusted to 50 c.c. Lead acetate solution (pH 7·25; 10 c.c.), prepared as described by Barker, Bourne, and Peat (*loc. cit.*), was added and the precipitated lead–protein complex was collected in the centrifuge. The precipitate was stirred with 0·2N-sodium hydrogen carbonate (50 c.c.) for 5 minutes, and a stream of carbon dioxide was then passed through the suspension for $2\frac{1}{2}$ minutes. The insoluble residue was removed by the centrifuge and discarded. To the supernatant liquid, ammonium sulphate solution (pH 7·0; 50 g./100 c.c. of solution) was added, the final concentration of this salt being 25 g./100 c.c. The small amount of proteinaceous material thus precipitated was collected in the centrifuge and re-dissolved in 0·2M-citrate buffer (pH 7·0; 30 c.c.).

Standard Digest of Amylose and Q-Enzyme.—The standard digest was composed of amylose solution (7·5 c.c.; containing 14 mg. of amylose), maltose solution (1·5 c.c.; containing 3·0 mg. of maltose), 0·2M-citrate buffer (pH 7·0; 2·5 c.c.), and Q-enzyme solution (2·5 c.c.), making a total volume of 14 c.c. The amylose solution was prepared by dissolving the polysaccharide in sodium hydroxide and neutralising the solution with sulphuric acid, as described by Barker, Bourne, and Peat (*loc. cit.*). The digest was incubated at 25°; at intervals, aliquot portions (1 c.c.; equivalent to 1 mg. of amylose) were stained with iodine (2 mg.) and potassium iodide (20 mg.) in a final volume of 100 c.c. and the absorption values were measured. Other portions (5 c.c.) were subjected to the Shaffer–Hartmann determination of reducing sugar (*J. Biol. Chem.*, 1921, **45**, 377). This reagent is insensitive to small amounts of sugar (<0·2 mg. of glucose and <0·4 mg. of maltose) and it was for this reason that maltose was introduced into the digest. A control digest, containing water (7·5 c.c.) in place of the amylose solution, was simultaneously incubated at 25°, and its reducing power was measured at intervals. The difference between the reducing values of the two digests was a measure of the reducing sugar liberated by the action of the enzyme on amylose and is expressed as "apparent conversion into maltose."

Polysaccharide Synthesis by a Cell-free Extract.—The enzyme solution employed was that obtained when the disrupted flagellates were extracted with 0·02M-citrate buffer, as described in the standard method. Portions (2 c.c.) of the enzyme solution were incubated at 18° with 0·125M-solutions of various sugars in 0·2M-citrate buffer (pH 6·0, 8·0 c.c.). The sugars used were glucose, glucose-1 phosphate, maltose, and sucrose. After 36 hours, the digest containing the Cori ester gave a deep reddish-purple stain with iodine, whereas the other three digests did not give a stain.

Development of Optimum Conditions for the Isolation of Q-Enzyme.—(a) *Fractionation with ammonium sulphate.* The crude enzyme, extracted from a 4-l. mass culture grown in a synthetic

medium, was treated with lead acetate and the precipitate was eluted with sodium hydrogen carbonate solution, as described in the standard method. The eluate was treated with neutral ammonium sulphate solution (50 g./100 c.c.), giving successive concentrations of this salt of 18, 24, 30, and 37·5 g./100 c.c. Each precipitate was collected in the centrifuge and redissolved in 0·2M-citrate buffer (pH 7·0; 15 c.c.). The four enzyme solutions thus obtained were examined for Q-enzyme activity in standard digests containing potato amylose (B.V. 0·95), and for phosphorylase activity by Green and Stumpf's method (*J. Biol. Chem.*, 1942, **142**, 355). The results are recorded in the following table.

$(NH_4)_2SO_4$ concn. (g./100 c.c.)	Phosphorylase activity	Test for Q-enzyme: Fall (%) in A.V. (6800 Å) of amylose : 45 min.	6 h.	Apparent conversion into maltose (%) in 6 h
0—18	Nil	74·7	90·6	Nil
18—24	Nil	83·1	90·6	1·9
24—30	Slight	81·5	91·5	Nil
30—37·5	Nil	1·0	26·4	Nil

In order to obtain a good recovery of Q-enzyme with little or no contamination by phosphorylase, the fraction precipitated in the 0—25 g./100 c.c. range of ammonium sulphate concentrations was retained in the standard method.

(b) *Elution of the lead–protein complex.* The ruptured cells from a mass culture, grown in a synthetic medium (4 l.), were extracted with 0·02M-citrate buffer (pH 7·0; 50 c.c.), and the extract was divided into two equal portions, each of which was treated with lead acetate, as described in the standard method. One of the lead–protein complexes was extracted with 0·2N-sodium hydrogen carbonate (25 c.c.) in a stream of carbon dioxide (see the standard method), and the other with 0·2M-citrate buffer (pH 7·0; 25 c.c.). Each of the two extracts was treated with neutral ammonium sulphate solution (final concentration, 25 g./100 c.c.) and the proteinaceous precipitate was dissolved in 0·2M-citrate buffer (pH 7·0; 15 c.c.). The two enzyme solutions thus obtained were incorporated in standard digests containing potato amylose (B.V. 0·95); the changes in A.V. (6800 Å) and in reducing power are shown in the following table. It was observed that ammonium sulphate precipitated considerably more material from the citrate extract than it did from the sodium hydrogen carbonate extract.

Extraction by :	Test for Q-enzyme: Fall (%) in A.V. (6800 Å) in : 30 min.	2 h.	Apparent conversion into maltose (%) in 23 h.
0·2N-$NaHCO_3$	67·0	83·9	4·7
0·2M-Citrate buffer	82·8	89·4	8·4

(c) *Freeze-drying of Q-enzyme solutions.* The preceding experiment was repeated and extended. A sample (10 c.c.) of each of the final 0·2M-citrate solutions of Q-enzyme was freeze-dried and redissolved in water, the volume of the solution being adjusted to 10 c.c. The effect of the drying process on enzymic activity can be seen in the next table; these observations were confirmed in several other experiments.

Extraction by :	Freeze-drying	pH of final enzyme solution	Test for Q-enzyme: Fall (%) in A.V. (6800 Å) in : 40 min.	4·5 h.	Apparent conversion into maltose (%) in 21 h.
0·2N-$NaHCO_3$	Omitted	7·39	65·5	87·2	1·5
	Included	6·95	14·4	19·2	1·3
0·2M-Citrate buffer	Omitted	7·40	77·8	87·2	4·3
	Included	6·79	73·4	87·6	4·3

A More Detailed Examination of the Action of Q-Enzyme on Amylose.—A solution of Q-enzyme, prepared by the standard method, was incorporated in a standard digest, which was prepared on thrice the usual scale; the polysaccharide employed was potato amylose (B.V. 0·95). The digest was incubated at 25° and the course of the enzyme action was followed by measuring the changes in the reducing power and iodine-staining properties, as in the standard digest. The complete absorption spectra of the polysaccharide–iodine stains in the visible region were measured. The results are shown graphically in Figs. 1 and 3. In a second experiment, in which a different enzyme preparation was employed, similar results were obtained.

Optimum Temperature for the Action of Q-Enzyme on Amylose.—A sample of Q-enzyme, prepared by the standard method, was incorporated in six standard digests, the polysaccharide being potato amylose (B.V. 0·95). The digests were incubated at temperatures ranging from 10° to 35°, and, after 32 and 75 minutes, respectively, withdrawals (1·0 c.c.) were made for the

determination of A.V. (6800 Å). The results are shown in the following table; when plotted, they reveal the optimum temperature to be 25—27°. That the enzyme employed in this experiment was substantially free from amylases was shown by the fact that, at 25° and pH 7·0, it effected an 84% decrease in A.V. (6800 Å) while the " apparent conversion into maltose " was only 1·3%.

Temp. of incubation		10°	15°	20°	23°	30°	35°
Fall (%) in A.V. (6800 Å) in	32 min.	40·2	51·0	58·0	67·0	59·0	34·0
	75 min.	75·5	79·7	81·0	83·0	78·0	44·8

In a duplicate experiment, 0·2M-citrate buffer (pH 6·8) was employed, both in the digest and as a solvent for the enzyme. When plotted, the result given in the following table show that, under these conditions, the optimum temperature was 32—34°.

Temp. of incubation		19°	25°	30°	35°	39·5°
Fall (%) in A.V. (6800 Å) in	17 min.	41·5	52·1	57·8	58·9	42·8
	27 min.	65·4	69·7	74·2	72·3	53·2

Temperature optima of 28° and 32—33° were found on using 0·02M-citrate buffers of pH 7·2 and 6·8, respectively.

Optimum pH for the Action of Q-Enzyme on Amylose.—Q-Enzyme, prepared by the standard method, was incorporated in seven digests of the standard type, except that 0·2M-citrate buffer solutions of different pH values were employed; these buffers differed only in the amounts of sodium hydroxide which they contained. The digests were incubated at 25°, withdrawals (1·0 c.c.) being made after 45 and 90 minutes for the determination of A.V. (6800 Å). The results are shown in the next table; the pH values recorded are those of the complete digests. Below pH 5·0 protein was precipitated from the digests and no fall in A.V. (6800 Å) could be detected. The Q-enzyme sample employed effected, at pH 7·0 and 25°, an 89% fall in A.V. (6800 Å) with only 2·1% " apparent conversion into maltose."

pH value of digest		4·11	5·24	6·29	6·86	7·30	7·55	7·62
Fall (%) in A.V. (6800 Å) in	45 min.	0·0	13·3	52·1	69·6	74·6	72·4	72·4
	90 min.	0·0	29·8	75·5	83·0	85·0	83·5	83·5

Autocatalysis by Q-Enzyme during Polysaccharide Synthesis by Phosphorylase.—(a) *Isolation of phosphorylase.* The source of phosphorylase was a sample of fraction P2, which had been isolated several months earlier from potatoes and freeze-dried in 0·2M-citrate buffer (pH 6·0), as described by Barker, Bourne, Wilkinson, and Peat (*loc. cit.*). The enzyme was further purified by two fractional precipitations with neutral ammonium sulphate, the fraction which was precipitated between salt concentrations of 18 and 35 g./100 c.c. being retained at each stage. The product was dissolved in 0·2M-citrate buffer (pH 6·0; 20 c.c. per 100 c.c. of potato juice employed) and freeze-dried. When required, the free-flowing powder was dissolved in water (20 c.c. per 100 c.c. of potato juice employed).

(b) *Preparation of dipotassium glucose-1 phosphate.* The Cori ester was prepared from potato starch by phosphorolysis, as recommended by Sumner and Somers (*Arch. Biochem.*, 1944, **4**, 11).

(c) *Compositions of digests.* Three digests, having the compositions shown below, were incubated at 25°. The Q-enzyme solution used had been prepared by the standard method. At intervals, aliquot portions (2·0 c.c.) were withdrawn and inactivated with 6% trichloroacetic acid (2·85 c.c.). The precipitates were collected in the centrifuge, and the inorganic phosphate in each supernatant liquid was estimated by Allen's colorimetric method (*Biochem. J.*, 1940, **34**, 858), corrections being applied for traces of inorganic phosphate in the enzyme samples. The results are shown graphically in Fig. 4. Similar results were obtained in a second series of digests. In addition, it was shown that heat-inactivated Q-enzyme did not increase the rate of polysaccharide synthesis by phosphorylase.

Solution	Volume (c.c.) in digest: A	B	C
0·1M-Dipotassium glucose-1 phosphate	9·00	9·00	9·00
Phosphorylase	6·75	6·75	—
Q-Enzyme	6·75	—	6·75
0·2M-Citrate buffer (pH 7·0)	9·00	15·75	15·75

The authors express their gratitude to Dr. A. Lwoff of the Pasteur Institute for kindly supplying the culture of *Polytomella coeca* used in this work, and to Courtaulds' Scientific and Educational Trust and the Department of Scientific and Industrial Research for financial assistance to two of them (I. A. W. and A. B.).

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49. *The Conversion of Amylose into Amylopectin by the Q-Enzyme of* Polytomella coeca.*

By A. Bebbington, E. J. Bourne, and I. A. Wilkinson.

Samples of potato amylose have been treated with the Q-enzyme of *Polytomella coeca*, and the polysaccharide products, which stain red with iodine, have been isolated. The polysaccharides have been examined with regard to (*a*) the intensities of their iodine stains in both the visible and the ultra-violet region of the spectrum, (*b*) their behaviour when titrated potentiometrically with iodine, (*c*) their precipitability by thymol, and (*d*) their susceptibility to attack by β-amylase. In addition, they have been subjected to methylation and end-group assay, and also to molecular-weight determinations. By these methods they have been shown to be members of the amylopectin–glycogen class.

In the preceding paper it was shown that the organism *Polytomella coeca*, grown on a purely synthetic medium, contains an enzyme which is similar to potato Q-enzyme (Haworth, Peat, and Bourne, *Nature*, 1944, **154**, 236; Bourne and Peat, *J.*, 1945, 877; Bourne, Macey, and Peat, *J.*, 1945, 882) inasmuch as it converts amylose into a polysaccharide which stains red with iodine; this conversion involves the liberation of no more than traces of reducing sugar, and does not require the addition of mineral phosphate. We now report that larger amounts (*ca.* 2 g.) of potato amylose (B.V. 1·25) have been treated with three different samples of the protozoal Q-enzyme, until the values of A.V. (6800 Å) had fallen by 89, 81, and 90%, severally, and that the polysaccharide products (I, II, and III) have been isolated and examined. They have been shown to possess branched structures similar to those of amylopectin and glycogen.

Polysaccharides I, II, and III contained only very small amounts of non-carbohydrate impurities, for their protein contents were less than 0·5%, and they afforded less than 2·0% of ash on ignition. Moreover, the reducing powers of hydrolysates of the polysaccharides, determined by cuprimetric titration, corresponded to conversions into glucose of 96, 94, and 96%, respectively. The true conversions into glucose must have been slightly greater than these figures suggest, because it has been shown (Pirt and Whelan, *J. Sci. Food Agric.*, 1951, **2**, 224) that, when glucose itself is treated with sulphuric acid, under conditions similar to those obtaining during the hydrolysis of the polysaccharides, the reducing power of the solution falls to about 97% of its initial value. That glucose was, in fact, the only reducing sugar which was present in the hydrolysates of the polysaccharides was demonstrated by filter-paper chromatography; an aniline hydrogen phthalate spray revealed a single aldose component, having an R_F value identical with that of a reference glucose spot, and a resorcinol spray failed to detect a ketose sugar.

The behaviour of the three polysaccharides (I—III) towards iodine was examined in some detail; in qualitative tests they gave red stains, in contrast to the intense blue colour given by the parent amylose. Their blue values, determined by the methods of Hassid and McCready (*J. Amer. Chem. Soc.*, 1943, **65**, 1154) and of Bourne, Haworth, Macey, and Peat (*J.*, 1948, 924), were 0·14, 0·24, and 0·12, respectively, compared with 0·10 for waxy maize starch (Bourne and Peat, *J.*, 1949, 5) and 0·11 for the amylopectin component of the native starch of *Polytomella coeca* (Bourne, Stacey, and Wilkinson, *J.*, 1950, 2694). The light-absorption curves of the iodine-stained solutions showed peaks at 5200 Å; under the same conditions, waxy maize starch and potato amylose exhibit absorption maxima at 5200 and 6400 Å, respectively (Barker, Bourne, and Peat, *J.*, 1949, 1712). Polysaccharides II and III were compared with potato amylose and potato amylopectin in other experiments, designed to measure (*a*) the dependence of A.V. (6800 Å) on iodine–iodide concentration (Fig. 1), (*b*) the changes in potential which occur during titrations of the polysaccharides with iodine–iodide solutions (Fig. 2), and (*c*) the

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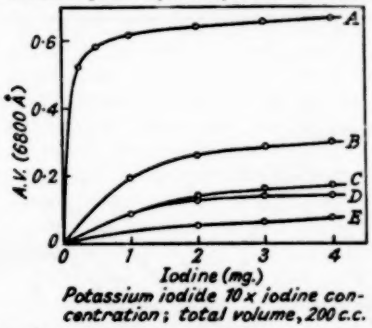
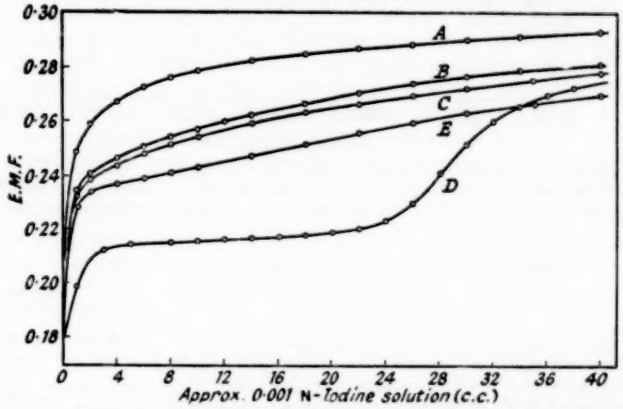
ultra-violet absorption spectra of their iodine-stained solutions (Fig. 3). All of these methods demonstrated that the polysaccharides under examination were markedly different from the parent amylose and were, in fact, very similar to potato amylopectin in their reactions with iodine. In support of this, they did not exhibit the property, characteristic of amyloses, of forming insoluble complexes with thymol (Tables 1 and 3). As was expected, since it represented an intermediate stage in the action of Q-enzyme on amylose, polysaccharide II showed less resemblance to potato amylopectin than did the other two polysaccharides.

FIG. 1. *Variation of A.V.* (6800 Å) *with iodine concentration.*



A, Potato amylose (*B.V.* 1·25; 1 mg.). *B, Polysaccharide II* (2 mg.). *C, Potato amylopectin* (*B.V.* 0·15; 2 mg.). *D, Polysaccharide III* (2 mg.). *E, Glycogen* (25 mg.).

FIG. 2. *The iodine titration of polysaccharide solutions.*



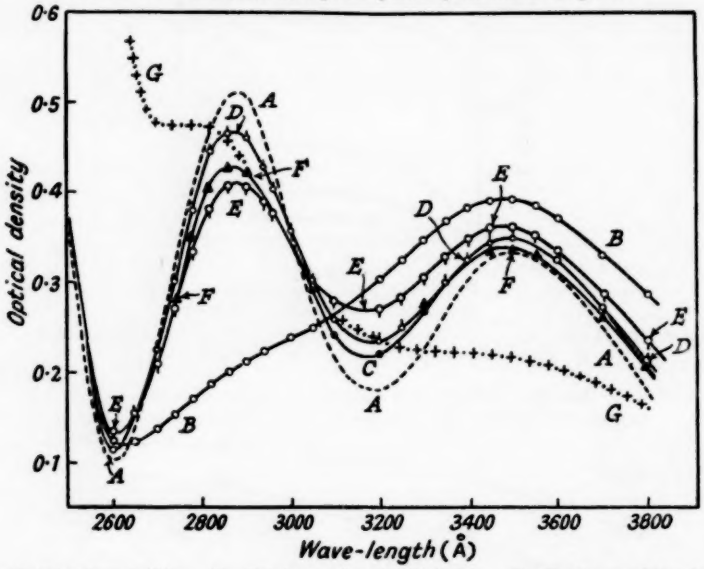
A, Iodine blank. B, Potato amylopectin (40 mg.). *C, Polysaccharide III* (40 mg.). *D, Potato amylose* (20 mg.). *E, Polysaccharide II* (40 mg.).

It is interesting to note the different effects which branched and unbranched structures have on the ultra-violet absorption spectrum of an iodine–iodide solution. In the absence of polysaccharide, the solution possesses two absorption peaks (at λ 2880 and 3500 Å), which have been attributed by Awtrey and Connick (*J. Amer. Chem. Soc.*, 1951, **73**, 1842) to the I_3^- ion. Potato amylopectin causes a slight decrease in the height of the peak at 2880 and a slight increase in that at 3500 Å (cf. Schlamowitz, *J. Biol. Chem.*, 1951, **190**, 519). On the other hand, potato amylose has a more pronounced influence on both peaks; indeed, the one at 2880 Å is eliminated completely.

There is a second respect in which the ultra-violet absorption spectra shown in Fig. 3 are important; this concerns Beckmann and Roger's claim (*J. Biol. Chem.*, 1951, **190**, 467) that potato Q-enzyme is an artifact, and that the red-staining product of its inter-

action with amylose is an amylose–soap complex, similar to that formed from amylose and potassium linoleate. There is already abundant evidence to be found in our earlier papers, published in collaboration with Professor S. Peat, that the active fraction isolated from potatoes is in fact an enzyme and not a salt of a fatty acid, and the claims made in those papers have been fully substantiated recently by Nussenbaum and Hassid (*J. Biol. Chem.*, 1951, **190**, 673). However, since Beckmann and Roger, in collaboration with Forster (*Amer. Chem. Soc. Symp. Abstracts*, September 1949), have themselves advocated the use of ultra-violet absorption spectra of the type described in order to distinguish

FIG. 3. *The ultra-violet absorption spectra of the iodine complexes.*



A, Iodine and potassium iodide. B, Potato amylose (B.V. 1·25). C, Potato amylopectin (B.V. 0·15) (coincident with D below ca. 3000 Å). *D, Polysaccharide III. E, Polysaccharide II. F, Synthetic polysaccharide (B.V. 0·22) from glucose-1 phosphate. G, Amylose–potassium linoleate complex.*

TABLE 1. *Characteristics of polysaccharides* I—III.

	Polysaccharide		
	I	II	III
Ash content (%)	0·8	1·4	2·0
Protein content (approx. %)	<0·5	<0·5	<0·5
Conversion (%) into glucose	96	94	96
Proportion (%) precipitated by thymol	—	4·0	1·2
Blue value	0·14	0·24	0·12
Wave-length (Å) of peak absorption of iodine stain in visible region	5200	5200	5200
Limiting conversion (%) into maltose by β-amylase	34	34	33
Blue value of limit dextrin	—	—	0·06
Average chain length	—	44	23, 39
Degree of polymerisation	—	—	128

between amylopectin and an amylose–soap complex, we wish to emphasise that in Fig. 3 there are very striking differences between the curves for polysaccharides II and III and potato amylopectin, on the one hand, and a red-staining amylose–potassium linoleate complex, on the other. In the same manner, a polysaccharide synthesised by Barker, Bourne, Peat, and Wilkinson (*J.*, 1950, 3022) from glucose-1 phosphate, by the agency of a mixture of potato phosphorylase and potato Q-enzyme, has been distinguished sharply from an amylose–soap complex. As supplementary evidence to support the contention that the Q-enzyme of *Polytomella coeca* is not a fatty acid, we have demonstrated its

extreme sensitivity to freeze-drying (see previous paper) and to inhibitors, such as mercuric chloride (Table 2).

Direct chemical evidence regarding the structures of polysaccharides II and III has been obtained by methylation and end-group assay. The two polysaccharides were methylated with sodium and methyl iodide in liquid ammonia, as described by Freudenberg and Boppel (*Ber.*, 1938, **71**, 2505), and hydrolysates of the resulting ethers were analysed quantitatively by filter-paper chromatography (cf. Hirst, Hough, and Jones, *J.*, 1949, 928). In each case, an aniline hydrogen phthalate spray revealed four components, three of which had R_F values identical with those of 2 : 3-dimethyl glucose, 2 : 3 : 6-trimethyl glucose, and 2 : 3 : 4 : 6-tetramethyl glucose, severally. The fourth spot had an R_F value slightly smaller than that of 2 : 3-dimethyl glucose, and was probably a second dimethyl glucose arising wholly or in part from incomplete methylation of the polysaccharide. Similar fourth spots have been found by Hirst, Hough, and Jones (*loc. cit.*) and by Barker, Bourne, and Wilkinson (*J.*, 1950, 3027) during examinations of hydrolysates of the methyl ethers of waxy maize starch and potato amylopectin. The relative proportions of the various methylated glucoses derived from polysaccharide II revealed that this polysaccharide had an average chain length of 44 units (Table 4). The methyl ether of polysaccharide III was divided into two fractions by extraction with ether; the average chain lengths of the larger and the smaller fraction were 23 and 39 units, respectively (Tables 5 and 6). Since the average chain length of the parent potato amylose, as determined by methylation and end-group assay, is *ca.* 200 units (Bourne, Fantes, and Peat, *J.*, 1949, 1109), it is clear that the action of the protozoal Q-enzyme on the linear polysaccharide had caused a marked decrease in chain length, the value finally reached, in the case of polysaccharide III, being very similar to that usually found for amylopectin.

The molecular size of polysaccharide III was determined by the method of Meyer and his colleagues (*J. Phys. Coll. Chem.*, 1949, **53**, 319), involving determination of the reducing end-group by treatment with an alkaline solution of 3 : 5-dinitrosalicylic acid. The degree of polymerisation was *ca.* 130 units (*M*, 21,000), which was appreciably higher than the average chain length of this polysaccharide, but lower than that of a natural amylopectin. However, if Q-enzyme is in fact a trans-glucosidase, then it is possible that any product of its action on amylose would not have a molecular weight higher than that of the linear parent polysaccharide. On this hypothesis, a product of higher molecular weight would result only when polysaccharide synthesis by phosphorylase is proceeding simultaneously with Q-enzyme action. This point will be examined further.

The behaviour of polysaccharides I, II, and III towards soya bean β-amylase furnished additional evidence that they possessed branched structures. However, the limiting conversions into maltose (33—34%) were somewhat lower than that (54%) observed when potato amylopectin (B.V. 0·15) was treated with the same enzyme sample. In addition, the B.V. of the limit dextrin, which was isolated only in the case of polysaccharide III, was 0·06, compared with 0·12 for that derived from potato amylopectin. These figures are more in keeping with those reported for glycogen (for summary, see Barker, Bourne, and Stacey, *J.*, 1950, 2884), the low limiting conversion of which has been attributed by Meyer and Jeanloz (*Helv. Chim. Acta*, 1943, **26**, 1784) to the inability of β-amylase to penetrate the more complex regions of the polysaccharide molecule. The same reason may apply in the present case, or, alternatively, the distribution of the branch points in polysaccharide III may be somewhat different from that in potato amylopectin (cf. Barker, Bourne, Peat, and Wilkinson, *loc. cit.*; Cori and Illingworth, *J. Biol. Chem.*, 1951, **190**, 679).

Experimental

Isolation of Polysaccharides formed from Potato Amylose by the Q-Enzyme of Polytomella coeca.—A sample of amylose (2·0 g.; B.V. 1·25), which had been prepared from potato starch by fractional precipitation with thymol (Bourne, Donnison, Haworth, and Peat, *J.*, 1948, 1687), was dissolved in warm 0·5N-sodium hydroxide (100 c.c.). The cooled solution was diluted with water (1 l.), neutralised with 0·5N-sulphuric acid, mixed with a solution of Q-enzyme in 0·2M-citrate buffer (pH 6·8; 200 c.c.), and diluted further to 2 l. The enzyme had been prepared from a mass culture (16 l.) of *Polytomella coeca* by the standard method described

in the preceding paper. The digest was incubated at 25°; at intervals, aliquot portions (1 c.c., equivalent to 1 mg. of amylose) were stained with iodine (2 mg.) and potassium iodide (20 mg.) in a total volume of 100 c.c. In addition, the reducing power of the digest was measured periodically by cuprimetric titration with the Shaffer–Hartmann reagent (*J. Biol. Chem.*, 1921, **45**, 377), a known amount of maltose being introduced during the determination in order to overcome the insensitivity of the reagent to small amounts of sugar (<0·4 mg. of maltose). When the A.V. (6800 Å) had reached an appropriate value, the digest was inactivated at 100° and the precipitated protein was removed by filtration. The filtrate and washings were concentrated under diminished pressure to 150 c.c. and dialysed for 48 hours. The polysaccharide product was precipitated with alcohol (500 c.c.), collected in the centrifuge, triturated with alcohol and then with ether, and finally dried in a vacuum over phosphoric oxide.

From three experiments of this type, in which different samples of Q-enzyme were employed, and in which the final values of A.V. (6800 Å) were about 11, 19, and 10% of the initial value, polysaccharides I, II, and III were isolated in yields of 65, 61, and 80%, respectively. In each case, the " apparent conversion into maltose " at the end of the incubation period was only 1—4%.

Purities of Polysaccharides I—III.—(a) *Ash contents.* Each polysaccharide (20 mg.) was heated in a platinum boat in a micro-muffle furnace until there was no further change in weight.

(b) *Products of acidic hydrolysis.* Each polysaccharide (40 mg.) was heated with 2N-sulphuric acid (1·0 c.c.) for 5 hours at 100° in a sealed tube. The hydrolysate was freed from inorganic material by means of an apparatus of the type described by Consden, Gordon, and Martin (*Biochem. J.*, 1947, **41**, 590). Part of the solution was examined for sugars by filter-paper chromatography; the solvent was the upper layer of a mixture of *n*-butanol (40%), ethanol (10%), water (49%), and ammonia (1%), and the spraying reagents were aniline hydrogen phthalate (Partridge, *Nature*, 1949, **164**, 443) and a 3% solution of resorcinol in 5% alcoholic hydrogen chloride. The former spray, which is more sensitive for aldoses than for ketoses, revealed only one component in each of the three hydrolysates, and this component had an R_F value identical with that of a glucose reference spot. The latter spray, which is more sensitive for ketoses, failed to detect any sugar component.

A second portion of each hydrolysate was examined, by filter-paper chromatography, for amino-acids, a saturated aqueous solution of phenol being used as the solvent and a 0·2% solution of ninhydrin in *n*-butanol as the spraying reagent. By comparison of the intensities of the spots with those obtained from amino-acid solutions of known concentration, it was shown that each of the three polysaccharides contained less than 0·5% of proteinaceous impurity. Moreover, negative reactions were obtained when the polysaccharides were tested for nitrogenous impurities by fusion with sodium and for proteins by treatment with Millon's mercuric nitrite reagent.

(c) *Degrees of conversion into glucose.* Polysaccharides I, II, and III were hydrolysed for 6 hours with hot 7% sulphuric acid, and the amounts of glucose produced were determined by cuprimetric titration, as described by Bourne, Donnison, Haworth, and Peat (*loc. cit.*). The conversions were 96, 94, and 96%, respectively.

Intensities of Polysaccharide–Iodine Stains.—Details of the methods employed for the determination of blue value (B.V.) and absorption value (A.V.) were given by Bourne, Haworth, Macey, and Peat (*loc. cit.*).

Potentiometric Titration of the Polysaccharides with Iodine.—The method employed was essentially that of Bates, French, and Rundle (*J. Amer. Chem. Soc.*, 1943, **65**, 142), as modified by Wilson, Schoch, and Hudson (*J. Amer. Chem. Soc.*, 1943, **65**, 1380). The polysaccharide sample (20 mg. in the case of amylose and 40 mg. in the case of a branched polysaccharide) was dispersed in 0·5N-potassium hydroxide (10 c.c.). The solution was neutralised (methyl-orange) with 0·5N-hydrochloric acid, mixed with 0·5N-potassium iodide solution (10 c.c.), and diluted with water to 100 c.c. A solution, which was approx. 0·001N in iodine, 0·05N in potassium iodide, and 0·05N in potassium chloride, was added to the polysaccharide solution in 1—2 c.c. portions. After each addition the potential was measured with the aid of a 3M-calomel cell and a bright platinum electrode. The results are shown graphically in Fig. 2.

Ultra-violet Absorption Spectra of Polysaccharide–Iodine Solutions.—(a) *The reference iodine–potassium iodide solution.* The absorption spectrum of a solution containing iodine (0·001%) and potassium iodide (0·01%), measured in a " Unicam " spectrophotometer with 10-mm. quartz cells, is shown in Fig. 3.

(b) *Modifications of the reference curve by various polysaccharides.* The polysaccharide (25 mg.) was suspended in water (5 c.c.), and N-sodium hydroxide solution (1 c.c.) was added.

The suspension was warmed until dissolution was completed, cooled, neutralised with 0·5N-sulphuric acid, and diluted to 25 c.c. A portion (5 c.c.) of the solution was stained with iodine (1 mg.) and potassium iodide (10 mg.) in a final volume of 100 c.c. The absorption spectrum of this solution was determined as in (a). The results obtained with various polysaccharides are shown in Fig. 3. In a control experiment, in which the polysaccharide was omitted, it was demonstrated that, under these conditions, sodium sulphate alone had a negligible effect on the absorption spectrum of the iodine–iodide solution. The amylose–potassium linoleate solution referred to in Fig. 3 contained amylose (5 mg., dissolved as before), potassium linoleate (17 mg.), and sufficient of the iodine–iodide solution to give a permanent red stain; excess of iodine–iodide solution produced a blue colour.

Inhibition of Q-Enzyme by Mercuric Chloride.—A sample of Q-enzyme, which had been prepared by the standard method from a mass culture of *Polytomella coeca*, was incorporated in four digests, each of which contained potato amylose (B.V. 1·25; 20 mg.), 0·02M-citrate buffer (pH 6·8; 6·0 c.c.), and Q-enzyme solution [containing the enzyme from a 400 c.c. culture, dissolved in 0·02M-citrate buffer (pH 6·8; 2·0 c.c.)] in a total volume of 20 c.c. In addition, three of the digests contained mercuric chloride, the concentrations being 0·1, 0·01, and 0·001%, respectively. The digests were incubated at 25°; at intervals aliquot portions (1 c.c.) were stained with iodine (2 mg.) and potassium iodide (20 mg.) in a total volume of 100 c.c. for the measurement of A.V. (6800 Å). The results are given in Table 2. The two digests containing

TABLE 2.

Concn. of $HgCl_2$ in digest (%)		Nil	0·001	0·01	0·1
Fall (%) in A.V. (6800 Å) in	45 min.	67·1	0·0	0·0	0·0
	100 min.	87·6	0·9	1·7	2·6

the highest concentrations of mercuric chloride developed a slight turbidity; the other two remained clear.

β-*Amylolysis of Polysaccharides* I—III.—The limiting conversions into maltose recorded in Table 1 were determined by the method of Barker, Bourne, Peat, and Wilkinson (*loc. cit.*), which was based on cuprimetric titration. The β-amylase employed had been isolated from soya beans (Bourne, Macey, and Peat, *loc. cit.*).

Isolation of the Limit Dextrin from Polysaccharide III.—The polysaccharide (200 mg.) was incubated at 20° with M-acetate buffer (pH 7·0; 10 c.c.), N-acetic acid (10 c.c.), and 0·2% β-amylase solution (20 c.c.) in a total volume of 100 c.c. When the digest, which had pH 4·7, attained constant reducing power (equivalent to a 33% conversion into maltose), it was heated to 100°, and the precipitated protein was removed by filtration. The filtrate was dialysed for 4 days and concentrated to 30 c.c. Alcohol (3 vols.) was added to precipitate the dextrin, which was collected in the centrifuge, ground with alcohol, then with ether, and dried at 60° in a vacuum over phosphoric oxide. The product (100 mg.) had B.V. 0·06 and contained 0·9% of ash.

In a second experiment, potato amylopectin (B.V. 0·15; 2·50 g.) was treated with the same enzyme sample. The limiting conversion into maltose was 54%. The limit dextrin (0·90 g.) had B.V. 0·12 and contained 0·8% of ash.

Treatment of Polysaccharides II *and* III *with Thymol.*—The polysaccharides were dissolved in alkali, neutralised, and treated with thymol according to the procedure of Barker, Bourne, Peat, and Wilkinson (*loc. cit.*); the results are given in Table 3.

Methylation of Polysaccharides II *and* III.—The polysaccharides were methylated with sodium and methyl iodide in liquid ammonia, as described by Freudenberg and Boppel (*loc. cit.*). The

TABLE 3. *Treatment of polysaccharides* II *and* III *with thymol.*

Polysaccharide	Fraction pptd. by thymol	
	Yield (%)	B.V.
II	4·0	0·17
III	1·2	0·07

modifications introduced by Barker, Bourne, and Wilkinson (*loc. cit.*) in the method used for the isolation of the products were again employed.

(a) *Methylation of polysaccharide* II. The polysaccharide (758 mg.) was treated eight times with the methylating reagents [one sixth of the quantities employed by Freudenberg and Boppel (*loc. cit.*)]. The crude product obtained when the ammonia was removed was stirred with water (200 c.c.), a small amount of insoluble material being separated by the centrifuge and discarded.

After being dialysed in " Cellophane " until iodide ions could no longer be detected by the silver nitrate test, the methylated polysaccharide was freeze-dried. The residue was extracted with ether for 3 hours (Soxhlet). The ether-insoluble portion (650 mg.) had OMe, 44·5% (Calc. for trimethyl starch : OMe, 45·6%). Evaporation of the ethereal extracts afforded a small residue (70 mg.) having OMe, 35·0%; this was not examined further.

(b) *Methylation of polysaccharide* III. The polysaccharide (616 mg.) was treated 16 times with the methylating reagents, and the product (428 mg.) was isolated as in (a). The ether-insoluble portion (209 mg.; fraction A) had OMe, 40·0% and ash content, 4·0%. The ethereal solution was concentrated to a syrup, to which chloroform (2 c.c.) was added. After the removal of some insoluble material, light petroleum (b. p. 100—120°; 40 c.c.) was added. The precipitate (80 mg.; fraction B) had OMe, 45·1%.

Analysis of the Hydrolysates of the Methylated Polysaccharides by Filter-paper Chromatography.—The hydrolysates were prepared and analysed by a method which was essentially that used by Flood, Hirst, and Jones (*J.*, 1948, 1679) and by Hirst, Hough, and Jones (*loc. cit.*). The methylated polysaccharide (*ca.* 60 mg.) was heated with 4% methanolic hydrogen chloride (1·0 c.c.) for 8 hours in a sealed tube at 100°. The glucosides produced were hydrolysed with 4% hydrochloric acid (5·0 c.c.) at 100° for 5 hours. The hydrochloric acid was removed by treatment with silver carbonate.

The components of the neutral hydrolysate were determined by filter-paper chromatography, the modifications introduced by Barker, Bourne, and Wilkinson (*loc. cit.*) being included. The blank strips used for the determination of reducing substances in the paper were cut from a second sheet of the same filter-paper which had been irrigated with the solvent and on which no hydrolysate spots had been placed. The sizes and positions from which the blank strips were cut were identical with those of the corresponding strips from the main chromatogram.

(a) *Analysis of methylated polysaccharide* II. The chromatogram obtained from a hydrolysate of methylated polysaccharide II showed four components which stained with aniline hydrogen phthalate. Three of these had R_F values identical with those of 2 : 3-dimethyl glucose, 2 : 3 : 6-trimethyl glucose, and 2 : 3 : 4 : 6-tetramethyl glucose, used as standards on the same chromatogram. The fourth component had an R_F value slightly smaller than that of 2 : 3-dimethyl glucose and appeared to be a second dimethyl glucose. Owing to the close proximity of the two dimethyl glucose spots, it was not possible to determine them separately and the figures quoted for the dimethyl glucose fraction in Table 4 represent the sum of both components. The mean value for the molecular proportion of 2 : 3 : 4 : 6-tetramethyl glucose corresponded to an average chain length of 44 glucose units.

TABLE 4. *End-group assay of methylated polysaccharide* II.

	Molecular composition (%) indicated by assay number				
Probable derivative of glucose	I	II	III	IV	Mean
Dimethyl fraction	2·37	2·60	2·31	2·84	2·53
2 : 3 : 6-Trimethyl	95·2	94·6	95·8	95·2	95·2
2 : 3 : 4 : 6-Tetramethyl	2·48	2·76	1·89	1·99	2·28

(b) *Analysis of methylated polysaccharide* III (*fraction A*). A chromatogram of a hydrolysate of methylated polysaccharide III (fraction A) showed four components having R_F values identical with those described in (a). The results obtained in three independent analyses are shown in Table 5. The results corresponded to an average chain length of 23 glucose units.

(c) *Analysis of methylated polysaccharide* III (*fraction B*). Chromatographic examination of a hydrolysate of methylated polysaccharide III (fraction B) revealed four components having R_F values identical with those reported in (a). Because of the small amount of the

TABLE 5. *End-group assay of methylated polysaccharide* III (*fraction A*).

	Molecular composition (%) indicated by assay no.			
Probable derivative of glucose	I	II	III	Mean
Dimethyl fraction	4·33	4·61	4·49	4·48
2 : 3 : 6-Trimethyl	91·2	91·0	91·1	91·1
2 : 3 : 4 : 6-Tetramethyl	4·49	4·41	4·43	4·44

polysaccharide ether available, only two end-group determinations could be made; these are shown in Table 6. The mean value for the molecular proportion of 2 : 3 : 4 : 6-tetramethyl glucose corresponded to an average chain length of 39 glucose units.

The Molecular Weight of Polysaccharide III.—A determination of the molecular size of one of the synthetic polysaccharides (III) was made by a method which was essentially that of Meyer, Bernfeld, Boissonnas, Gürtler, and Noelting (*J. Phys. Coll. Chem.*, 1949, **53**, 319). It involved the colorimetric estimation of the reducing end-group with 3 : 5-dinitrosalicylic acid in alkaline solution.

TABLE 6. *End-group assay of methylated polysaccharide* III (*fraction B*).

Probable derivative of glucose	Molecular composition (%) indicated by assay no. I	II	Mean
Dimethyl fraction	4·90	5·97	5·44
2 : 3 : 6-Trimethyl	92·5	91·5	92·0
2 : 3 : 4 : 6-Tetramethyl	2·63	2·49	2·56

The polysaccharide (51·8 mg.) was suspended in water (2 c.c.), and complete solution attained by the addition of 6N-sodium hydroxide solution (1 c.c.). An aliquot portion (1 c.c.) of a 1·5% solution of the reagent (3 : 5-dinitrosalicylic acid in 0·2N-sodium hydroxide solution) was added, and the mixture was heated at 65° for 30 minutes. After cooling, the volume was adjusted to 25 c.c. with water, and the intensity of the colour was compared with that of a blank (polysaccharide absent), the Spekker photoelectric absorptiometer being used with a green filter and 4-cm. cells. In order to correct for any turbidity due to the polysaccharide, a blank containing polysaccharide and alkali, but no 3 : 5-dinitrosalicylic acid, was treated as described above, and its absorption measured. The reagent had been previously standardised with solutions of maltose hydrate, so that the degree of polymerisation of the polysaccharide could be calculated directly from its absorption and the weight of maltose exhibiting the same absorption. A degree of polymerisation of 128 was obtained, corresponding to a molecular weight of approximately 21,000 for polysaccharide III.

The authors are indebted to Professor M. Stacey, F.R.S., for his interest in this work, and to Courtaulds' Scientific and Educational Trust and the Department of Scientific and Industrial Research for financial assistance to two of them (I. A. W. and A. B.).

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NOTES.

50. *The Distribution of Quinol between Ether and Water.*

By R. A. ROBINSON.

THE distribution coefficient of quinol between ether and water does not seem to be known with certainty; thus, the International Critical Tables (1928, vol. III, p. 428) give 2·7 as the ratio at 15° of the volume concentration in the ethereal layer to that in the aqueous layer, a figure based on Pinnow's data (*Z. anal. Chem.*, 1911, **50**, 162) although Seidell ("Solubilities of Organic Compounds," 3rd Edn., 1941, vol. II, p. 400) remarks that "the terms in which the concentration is expressed (by Pinnow) are not stated." A graph given by Baumbach (*J. Soc. Motion Picture Engineers*, 1939, **33**, 517) indicates a coefficient of 1·6 at 22°, said to be independent of concentration, contrary to the finding of Pinnow. On the other hand, extrapolation of Walker, Collett, and Lazzell's data (*J. Phys. Chem.*, 1931 **35**, 3259) indicates a ratio not far from 0·45 at room temperature on a weight basis and 0·32 on a volume basis.

The matter is of more than academic interest because quinone is often used as an oxidising agent and ether is used to extract the quinol formed in the reaction. If the product is to be used in animal feeding experiments, it is important to be assured that ether is an efficient solvent for the extraction.

I have measured the distribution coefficient at 25°, in an atmosphere of nitrogen, titrating the quinol with ceric sulphate and using ferrous phenanthroline as indicator

(Kolthoff and Lee, *Ind. Eng. Chem., Anal.*, 1946, **18**, 452). The following results are expressed in terms of g. of quinol per 100 g. of solution :

Ether	1·450	2·093	4·207	6·251	8·811	21·54	22·98
Water	0·513	0·697	1·222	1·659	2·092	3·738	3·901
$K_{obs.}$	2·83	3·01	3·44	3·77	4·21	5·77	5·89
$K_{calc.}$	2·83	3·00	3·47	3·86	4·25	5·73	5·88

$K_{calc.}$ is obtained from the empirical equation : $K_{calc.} = 2{\cdot}37\ (1 + 4{\cdot}17[Q]_W)$, where $[Q]_W$ is the concentration, in moles per 1000 g. of solution, of the aqueous layer. (If $K_{calc.}$ is expressed in terms of volume concentrations and $[Q]_W$ in g. of quinol per 5 c.c. of aqueous layer, this equation becomes $K_{calc.} = 1{\cdot}68 + 12{\cdot}7[Q]_W$, which compares with an equation given by Pinnow, $K_{calc.} = 1{\cdot}885 + 12{\cdot}6[Q]_W$, and suggests that he used g. per 5 c.c. of solution as his unit of concentration.) This equation is consistent with dimersiation in the ethereal layer, with an equilibrium constant, $K' = [Q]_E^2/[(Q)_2]_E$. If K° is the distribution coefficient of the monomer, it is easy to show that $K_{calc.} = K^\circ(1 + 2K^\circ[Q]_W/K')$, and this conforms with the experimental findings if $K^\circ = 2{\cdot}37$ and $K' = 1{\cdot}14$ moles per 1000 g. of solution.

Facile as this explanation may be, there is little evidence for the presence of double molecules of quinol in ether; indeed, Beckmann's b. p. measurements (*Z. physikal. Chem.*, 1889, **3**, 603) can be interpreted to imply only a slight association, far from the amount required by the above equation. For practical purposes, however, the distribution coefficient under the conditions prevailing when ether is used for extraction may be taken to be of the order of 3 on a wt. concentration basis and 2 if volume concentrations are used.

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51. *The Electrolytic Reductive Coupling of* p-*Dimethylamino-acetophenone and* p-*Methoxyacetophenone.*

By HAROLD A. LEVINE and MILTON J. ALLEN.

DURING an investigation of the mechanism of formation of pinacols from ketones at a mercury cathode surface by application of a controlled potential (Allen and Corwin, *J. Amer. Chem. Soc.*, 1950, **72**, 114; Allen, *Analyt. Chem.*, 1950, **22**, 804; *Trans. Electrochem. Soc.*, in the press) the reduction of a mixture of ketones was studied. Previous investigators (Laude and Wiemann, *Bull. Soc. chim.*, 1946, 256) have reported the formation of mixed pinacols when a mixture of ketones was reduced by metallic sodium. In extending these observations to pinacol formation by electrolytic reduction, a mixture of ketones was chosen such that a *quantitative* determination of the amount of mixed pinacol formation could be obtained. The reductive coupling of *p*-dimethylaminoacetophenone with *p*-methoxyacetophenone gave a product which could be completely separated from one of the starting materials and its neutral reduction products by solubility in dilute acid. Analysis of the basic fraction for methoxyl content afforded a direct measure of the yield of mixed pinacol. A check on these yields was obtained by comparing the analytical values found for nitrogen in the samples with values calculated for the appropriate mixture of mixed pinacol, 2-*p*-dimethylaminophenyl-3-*p*-methoxyphenylbutane-2 : 3-diol, and the symmetrical pinacol, 2 : 3-bis-(*p*-dimethylaminophenyl)butane-2 : 3-diol. These data, presented in the table, show satisfactory agreement. The presence of small amounts of basic products other than pinacols (cf. Allen and Corwin, *loc. cit.*) is, however, not excluded, but would not greatly affect the calculated end values.

Experimental.—The cell used was modified from that described by Allen and Corwin by addition of a 24/40 standard-taper joint for insertion of a reflux condenser. The anode chamber was a fine-porosity alundum crucible, 2·9 cm. in diameter and 8 cm. high, held in position by means of a carefully cut, tightly fitting rubber stopper. A cylindrical platinum anode was used. The cathode surface area was 23·5 cm.^{2}.

An equimolecular mixture of *p*-methoxyacetophenone (3·00 g.) and *p*-dimethylaminoacetophenone (3·26 g.) was dissolved in the appropriate catholyte as illustrated in the table. The solu-

Catholyte composition :	EtOH, 25 ml.; H_2O, 17 ml.; KOAc, 15 g.	EtOH, 15 ml.; 2N-HCl, 35 ml.	EtOH, 23 ml.; 2N-KOH, 30 ml.
Reference potential	− 1·97	− 1·06	− 1·86
Temperature	reflux, 83°	28—30°	reflux, 83°
Anolyte	40% K_2CO_3	Same as catholyte	Same as catholyte
Initial amperage	8·2	3·5	7·0
Final amperage	0·83	0·55	0·42
Reduction time, min.	18·0	50·0	24·0
Wt. of basic fraction, g.	3·65	3·79	3·93
OMe in basic fraction (%)	5·86	4·90	5·84
Mixed pinacol in basic fraction (%)	59·9	49·8	58·0
Yield of mixed pinacol (%)	34·7	30·2	37·3
N, calc. in basic fraction (%)	6·07	6·49	6·16
N, found (%)	5·93	6·04	6·00

tion was heated to boiling, and reduced electrolytically at a constant reference potential while reflux was maintained. Reduction was continued until the current remained approximately constant. The catholyte was evaporated at the water pump and made basic with concentrated potassium hydroxide solution where necessary, and the precipitated viscous oil washed with cold water several times by decantation. It was then dissolved in a mixture of N-hydrochloric acid (40 ml.) and ether (25 ml.). The aqueous solution was re-extracted with ether (25 ml.) and then made slightly basic with potassium hydroxide solution. The semi-solid precipitate was washed with water until the washings were neutral to litmus and dried *in vacuo* over phosphoric oxide. This material was analyzed for methoxyl and nitrogen contents.

Repeated fractional crystallization from *iso*propyl ether gave 2-*p*-dimethylaminophenyl-3-*p*-methoxyphenylbutane-2 : 3-diol as colourless needles, m. p. 121—122° (corr.; Kofler hot stage) (Found : C, 72·4; H, 8·3; N, 4·3. $C_{19}H_{25}O_3N$ requires C, 72·35; H, 8·0; N, 4·4%).

This work was carried out during the tenure by one of us (H. A. L.) of a Post-doctoral Research Fellowship of the National Cancer Institute.

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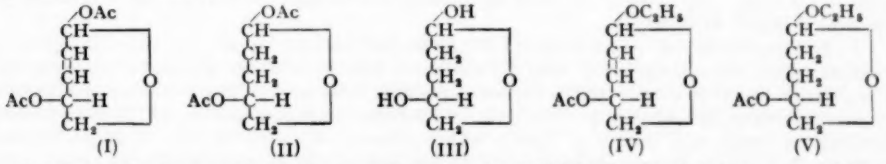
52. *Deoxy-sugars. Part XXIII.* An Extension of the Investigation of the Dische Reaction.*

By R. ALLERTON, W. G. OVEREND, and M. STACEY.

IT has been concluded that the presence of 2-deoxyribose in deoxypentose nucleic acids needs confirmation by methods (see Chargaff, Vischer, Doniger, Green, and Misani, *J. Biol. Chem.*, 1949, **177**, 405) alternative to the Dische diphenylamine test (*Mikrochem.*, 1930, **8**, 4) before its presence can be unequivocally accepted. Recently we have been engaged in preparing 2 : 3-dideoxy-hexose (Laland, Overend, and Stacey, *J.*, 1950, 738) and -pentose derivatives and, since the Dische reaction is given by 2-deoxypentoses generally, it was considered of interest to determine whether 2 : 3-dideoxypentoses gave any colour when added to the reagent.



(I) (II) (III) (IV) (V)

Catalytic hydrogenation of 1 : 4-diacetyl 2 : 3-didehydro-2 : 3-dideoxy-L-arabinose (I) afforded 1 : 4-diacetyl-2 : 3-dideoxy-L-ribose (II). This on deacetylation yielded syrupy 2 : 3-dideoxy-L-ribose (III) which readily yielded a dibenzyl mercaptal on treatment with toluene-ω-thiol and concentrated hydrochloric acid. 4-Acetyl αβ-ethyl 2 : 3-dideoxy-L-riboside (V) was prepared from 4-acetyl 2 : 3-didehydro-2 : 3-dideoxy-αβ-ethyl-L-arabinoside (IV) by the method of Gehrke and Aichner (*Ber.*, 1927, **60**, 918), followed by catalytic hydrogenation.

* Part XXII, *J.*, 1951, 2062.

Standardised quantitative Dische tests were carried out with 2 : 3-dideoxyribose and derivatives thereof, and also with intermediates obtained from the syntheses. In their quantitative Dische tests, Deriaz, Stacey, Teece, and Wiggins (*J.*, 1949, 1222) heated the reactants together for 3·25 minutes. It has been shown, however, that if the heating is prolonged to 15 minutes no further development of colour results after this period, and that by heating for this time better controls can be maintained Consequently we have adopted the latter conditions in preference to those described by Deriaz *et al.* (*loc. cit.*) (cf. Overend, *J.*, 1951, 1484; Woodhouse, *Brit. J. Cancer*, 1949, **3**, 510). It was found that, although a blue colour is produced when Dische reagent is added to 2 : 3-dideoxyribose, and also to its acetate and αβ-ethylglycoside, this is very weak and in no way comparable in intensity with the colour developed with 2-deoxyribose. Intermediates having an ethylenic linkage between $C_{(2)}$ and $C_{(3)}$ behave similarly to arabinal and diacetyl arabinal and give strong positive Dische tests.

For comparative purposes β-methyl-3-deoxy-L-xyloside (riboside) was prepared from 2 : 3-anhydro-β-methyl-L-riboside by essentially the method of Todd and Mukherjee (*J.*, 1947, 969). Hydrolysis afforded 3-deoxy-L-xylose (-L-ribose) which readily yielded a dibenzyl mercaptal. With the Dische reagent 3-deoxy-L-xylose and its glycoside gave blue colours similar to that obtained with 2 : 3-dideoxyribose.

In the preparation of 2-deoxyribose and 3-deoxyxylose derivatives from 2 : 3-anhydromethylriboside, a negative Dische test has frequently been taken as indicative of the absence of 2-deoxyribose in the reaction products (cf. Stacey, Kent, and Wiggins, *J.*, 1949, 1232; Davoll, Lythgoe, and Trippett, *J.*, 1951, 2230). Although it is now shown that a blue colour can be obtained when Dische reagent is added to 3-deoxyxylose and its derivatives, it must be emphasized that to obtain this colour it is necessary to use a much higher concentration of the sugar than is usual with 2-deoxyribose, and to heat for a much longer time to ensure onset of formation of the colour. It can be concluded that the diphenylamine colour reaction for 2-deoxypentoses is relatively non-specific and needs careful control when used in quantitative estimations.

Experimental.—*3-Deoxy-L-xylose* (*-L-ribose*). Prepared essentially by the method of Todd and Mukherjee (*loc. cit.*), the deoxy-sugar (0·114 g.) was shaken with toluene-ω-thiol (0·22 c.c.) and concentrated hydrochloric acid (0·22 c.c.); isolation in the usual manner gave *3-deoxy-L-xylose dibenzyl mercaptal* (0·21 g., 67%) as a yellow oil, b. p. 204—220°/0·008 mm., $[\alpha]_D^{21}$ +18·2° (*c*, 0·77 in chloroform) (Found: C, 62·9; H, 6·5; S, 17·7. $C_{19}H_{24}O_3S_2$ requires C, 62·7; H, 6·6; S, 17·6%).

1 : 4-Diacetyl 2 : 3-dideoxy-L-ribose. 1 : 4-Diacetyl 2 : 3-didehydro-2 : 3-dideoxy-L-arabinose (prepared by the method of Bergmann and Breuers, *Annalen*, 1929, **470**, 51) (5·1 g.) was shaken in methanol (40 c.c.) containing palladium on charcoal (1 g.) at room temperature in an atmosphere of hydrogen. When the uptake of hydrogen was complete the solution was filtered and the residue was well washed with methanol. The washings were added to the filtrate which was then concentrated to afford a syrupy residue. Distillation yielded *1 : 4-diacetyl 2 : 3-dideoxy-L-ribose* (4·14 g., 80·6%) as a colourless mobile liquid, b. p. 97—119°/0·01—0·03 mm., n^{21} 1·4425, $[\alpha]_D^{17}$ +34·4° (*c*, 0·69 in chloroform) (Found: C, 53·2; H, 6·8. $C_9H_{14}O_5$ requires C, 53·5; H, 6·9%).

2 : 3-Dideoxy-L-ribose. 1 : 4-Diacetyl 2 : 3-dideoxy-L-ribose (0·69 g.) was dissolved in methanol (30 c.c.) saturated at 0° with dry ammonia and the solution was kept at 0° for 4 days. The solution was then evaporated to dryness and ammonium acetate in the residue was removed by sublimation at 30—40°/0·005—0·01 mm. *2 : 3-Dideoxy-L-ribose* (0·28 g., 65%) was obtained as a viscous syrup, $[\alpha]_D^{19}$ +22·7° (*c*, 3·97 in water) (Found: C, 50·6; H, 8·7. $C_5H_{10}O_3$ requires C, 50·9; H, 8·5%). The deoxy-sugar (0·14 g.) was shaken for 20 minutes at room temperature with toluene-ω-thiol (2·2 mols., 0·304 c.c.) and concentrated hydrochloric acid (0·3 c.c.). Isolation in the usual manner afforded *2 : 3-dideoxy-L-ribose dibenzyl mercaptal* (0·22 g., 54·5%) as a yellow viscous liquid, b. p. 215—234°/0·009 mm. This crystallised after several days and was recrystallised from methanol–water; it then had m. p. 74°, $[\alpha]_D^{21}$ +20·4° (*c*, 0·88 in chloroform) (Found: C, 65·0; H, 6·9; S, 17·9. $C_{19}H_{24}O_3S_2$ requires C, 65·5; H, 6·9; S, 18·4%).

4-Acetyl 2 : 3-dideoxy-αβ-ethyl-L-riboside. 4-Acetyl 2 : 3-didehydro-2 : 3-dideoxy-αβ-ethyl-L-arabinoside (prepared according to the method of Gehrke and Aichner, *loc. cit.*) (2·08 g.) was dissolved in methanol (50 c.c.) containing palladium on charcoal (*ca.* 2 g.), and the mixture was

shaken in an atmosphere of hydrogen at room temperature. When the uptake of hydrogen was complete the solution was filtered and the filtrate evaporated to dryness. Distillation of the residue yielded *4-acetyl* 2 : 3-*dideoxy*-αβ-*ethyl*-L-*riboside* (1·37 g., 65%) as a colourless liquid, b. p. 85—95°/0·1 mm., n^{18} 1·4435, $[\alpha]_D^{17}$ +45·3° (*c*, 0·53 in chloroform) (Found : OEt, 24·2. $C_9H_{16}O_4$ requires OEt, 23·9%).

Quantitative Dische tests. The Dische reagent was prepared as described by Deriaz *et al.* (*loc. cit.*). The general procedure was to dissolve the sugar derivative in water (3 c.c.) and add Dische reagent (6 c.c.). The solution was heated at 100° for 15 minutes, then cooled at 0° for 10 minutes, and the intensity of the colour developed was measured by use of the Spekker photoelectric absorptiometer.

Intensity of Dische colours developed as shown by extinction coefficient (ε) *at* 5800 A.

Sugar derivative	ε	Sugar derivative	ε
2-Deoxy-D-ribose	3660	3-Deoxy-β-methyl-L-xyloside	125
β-Methyl-2-deoxy-D-ribopyranoside	4100	3-Deoxy-L-xylose	126
Diacetyl-L-arabinal	2540	3-Deoxy-L-xylose (heated for 30 mins.)	149·5
4-Acetyl *pseudo*-L-arabinal	2920	4-Acetyl αβ-ethyl-*pseudo*-L-arabinal (heated for 3·25 mins.)	1090
Diacetyl *pseudo*-L-arabinal	2560	4-Acetyl 2 : 3-dideoxy-αβ-ethyl-L-riboside (heated for 10 mins.)	216
1 : 4-Diacetyl 2 : 3-dideoxy-L-ribose	203		
2 : 3-Dideoxy-L-ribose	107		

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53. 8-*Methyl*-3 : 4-*benzopyrene.*

By J. M. L. CAMERON, J. W. COOK, and (MISS) R. SCHOENTAL.

8-METHYL-3 : 4-BENZOPYRENE has been synthesised from an available intermediate, in order to extend the range of 3 : 4-benzopyrene derivatives tested for carcinogenic activity. 1 : 2 : 3 : 12-Tetrahydro*meso*benzanthrene-3-β-propionic acid-A [prepared as described by Cook, Ludwiczak, and Schoental (*J.*, 1950, 1118)], was cyclised with anhydrous hydrogen fluoride. The product, purified by chromatography on alumina from benzene–light petroleum, gave not only the keto-octahydrobenzopyrene-A described by these authors, but also the more strongly adsorbed 8-*keto*-1 : 2 : 8 : 9 : 10 : 11-*hexahydro*-3 : 4-*benzopyrene* which formed canary-yellow leaflets (from benzene), m. p. 209—210° (Found : C, 87·9; H, 6·1. $C_{20}H_{16}O$ requires C, 88·2; H, 5·9%). A solution of this ketone (0·5 g.) in benzene (15 c.c.) was added to a stirred Grignard solution, prepared from magnesium turnings (2 g.) and methyl iodide (10 c.c.) in dry ether (50 c.c.). The mixture was kept overnight, then boiled for an hour, and decomposed with ice and ammonium chloride. The product was purified by passage of its benzene solution through a column of alumina, and crystallised from benzene–light petroleum and then acetone. 8-*Methyl*-1 : 2 : 10 : 11-*tetrahydro*-3 : 4-*benzopyrene* formed yellowish needles, m. p. 162—163° (Found : C, 93·4; H, 7·0. $C_{21}H_{18}$ requires C, 93·3; H, 6·7%). Dehydrogenation was effected by boiling a solution of this hydrocarbon (300 mg.) in 1-methylnaphthalene (5 c.c.) with palladium black (40 mg.) under nitrogen for 10 hours. The solution was diluted with light petroleum and passed through a column of alumina. The main, yellowish, band was cut and eluted with benzene, giving 8-*methyl*-3 : 4-*benzopyrene* as yellow prisms (from benzene), m. p. 192—193° (Found : C, 94·6; H, 5·3. $C_{21}H_{14}$ requires C, 94·7; H, 5·3%). The *picrate* formed dark brown needles (from benzene), m. p. 205° (decomp.) (Found : C, 65·55; H, 3·5; N, 8·15. $C_{21}H_{14}.C_6H_3O_7N_3$ requires C, 65·45; H, 3·5; N, 8·5%), and the *s.*-trinitrobenzene *complex* crystallised from benzene in red needles, m. p. 233° (decomp.) (Found : N, 8·5. $C_{21}H_{14}.C_6H_3O_6N_3$ requires N, 8·8%).

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54. *Organic Fluorides. Part XI.* Fluorination of Heterocyclic Compounds with Cobalt Trifluoride.*

By R. Montgomery and F. Smith.

The fluorination of heterocyclic compounds has received very little attention, and appears to lead to extensive degradation. Fluorination of pyridine by electrolysis in anhydrous hydrogen fluoride (Simons *et al.*, *J. Electrochem. Soc.*, 1949, **95**, 47) produced perfluoropentane and nitrogen trifluoride, and that of 2 : 6-lutidine (Haszeldine, *J.*, 1950, 1638), by means of either a catalytic or a cobalt trifluoride procedure, afforded a small amount of a compound believed to be perfluoro-2 : 6-dimethylpiperidine, the major portion of the products boiling below room temperature. Similar results have been obtained in the fluorination of quinoline, only a small amount of perfluorodecahydroquinoline being isolated (Haszeldine and Smith, unpublished). However, the nitrogen linkages in the chlorinated products of triphenylamine and *N*-phenylcarbazole were intact after treatment with both silver difluoride and antimony pentafluoride in the liquid phase (Stilmar, Struve, and Wirth, *Ind. Eng. Chem.*, 1947, **39**, 348), as also were those from the vapour-phase fluorination of several tertiary alkylamines with cobalt trifluoride (Haszeldine, *Research*, 1950, **3**, 430). A preliminary study of the fluorination of 2-methylindole with cobalt trifluoride in a rotating reactor (Massingham, Montgomery, Smith, and Thomas, *J. Appl. Chem.*, in the press) showed that, as with 2 : 6-lutidine and pyridine, the compound underwent extensive fragmentation; the main product was a mixture of perfluorohydrocarbons but a small amount of fully fluorinated material was obtained which still contained nitrogen. Fission of the oxygen–carbon linkage often, but not always, occurs on fluorination of diphenyl ether and its derivatives (Fowler *et al.*, *Ind. Eng. Chem.*, 1947, **39**, 292; Stilmar *et al.*, *loc. cit.*).

We have now shown that fluorination of thionaphthen in the vapour phase with cobalt trifluoride leads to complete elimination of the sulphur. The principal product, perfluoroethyl*cyclo*hexane, was accompanied by a small amount of perfluoro*cyclo*hexane and a high-boiling polymeric material, probably similar to those high-boiling fluorocarbons produced when hydrocarbons are subjected to fluorination under similar conditions (Haszeldine and Smith, *J.*, 1940, 2689). In no fraction could sulphur be detected, and it seemed most probable that sulphur hexafluoride was formed but could not be isolated under the experimental conditions (cf. Neudorffer, *Compt. rend.*, 1950, **231**, 1070).

In general, therefore, it would seem that the principal reaction in the fluorination of compounds containing nitrogen, oxygen, or sulphur is one of fission with the elimination of these elements and the fluorination of the hydrocarbon fragments. The extent of this degradation is dependent upon the method of fluorination and appears to be capable of modification by a preliminary stabilisation by chlorination; it is also probable that certain perfluoroheterocyclic nitrogen compounds could be obtained by use of a mild fluorinating agent, such as cobalt trifluoride, since the bond energies for C–N and N–F are 48·6 and 68·8 kcal. per mole, respectively (Pauling, "The Nature of the Chemical Bond," Cornell Univ. Press, 1940). Similarly, the bond energies for C–S and S–F, being 50·1 and 84·8 kcal. per mole, respectively (Skinner, *Trans. Faraday Soc.*, 1945, **41**, 645), will necessitate a new method of fluorination for the preparation of perfluoro-heterocyclic sulphur compounds.

Experimental.—Fluorination of thionaphthen with cobalt trifluoride. Thionaphthen (75 g.), in three portions, was passed at 10—15 g./hour over cobalt trifluoride in a vertical reactor heated to 330—390°. The effluent vapours were condensed in traps cooled in solid carbon dioxide–ethanol, and the product was washed with water. The crude product (94·2 g.) was extracted with absolute alcohol (Haszeldine and Smith, *J.*, 1950, 3617) and the alcohol-insoluble material (71·3 g.) was distilled, five fractions being collected (total weight 61·8 g.) with a total b. p. range up to 245°. Each of these fractions was found by sodium fusion to contain fluorine but in no case was sulphur detected. After treatment of each fraction with uranium hexa-

* Part X, *J. Appl. Chem.*, in the press.

fluoride in the usual way in order to effect complete fluorination (*idem, ibid.*, p. 2689) further distillation yielded fractions: (i) 1·6 g., b. p. 48—55°, m. p. 51°; (ii) 2·8 g., b. p. 55—96°; (iii) 24·6 g., b. p. 98—100°, d_4^{25} 1·827, d_4^{30} 1·813, η_i^{25} 17·19 millipoises, η_i^{30} 15·70 millipoises, n_D^{16} 1·289 (Found: F, 77·0. Calc. for C_6F_{14}: F, 76·0%); (iv) 9·1 g., b. p. 100—110°; (v) 2·0 g., b. p. 110—245°.

Fraction (i) was identified by m. p. and mixed m. p. as perfluoro*cyclo*hexane. Fraction (iii) was identical in physical characteristics with perfluoroethyl*cyclo*hexane, the constants for an authentic sample of which were: b. p. 101°; d_4^{25} 1·826, d_4^{30} 1·812; η_i^{25} 17·42 millipoises, η_i^{30} 15·76 millipoises; n_D^{15} 1·286. [The viscosities were determined in an Ostwald viscometer with water as reference liquid, so the values obtained differ from those quoted (*idem, ibid.*), for which benzene was used as reference liquid.] Fractions (ii) and (iv) were mixtures and fraction (v) was a viscous oil.

Fluorination of 2-methylindole. 2-Methylindole (170 g.), in three aliquots, was passed at rates of 15—35 g./hour over cobalt trifluoride in a rotating reactor (Massingham *et al.*, *loc. cit.*) heated to 336—390°. The effluent vapours were treated as described above, yielding a crude product (129·4 g.) containing alcohol-insoluble material (106·4 g.). This crude fluorocarbon mixture was treated with uranium hexafluoride in the usual way and repeatedly fractionally distilled, the 11 fractions obtained having the properties shown below:

Fraction	Wt. (g.)	B. p.	n_D^{21}	d_4^{25}	Fraction	Wt. (g.)	B. p.	n_D^{21}	d_4^{25}
1	1·7	35—48°	<1·255	—	8	5·8	120—135°/	1·3376	2·000
2	5·4	48—57	1·255	1·682			12 mm.		
3	4·1	57—87	1·276	1·765	9	4·2	135—155/	1·3461	2·031
4	8·6	87—103	1·291	1·807 (20°)			12 mm.		
5	5·9	103—108	1·297	1·826	10	6·7	158—176/	1·3518	—
6	4·2	137—148	1·3089	1·879 (20°)			12 mm.		
7	5·7	156—176	1·3171	1·920	11	12	Residue		

Only fractions (5) and (10) gave a positive test for nitrogen. Fraction (2) is probably perfluoro*cyclo*hexane for which Bigelow (*J. Amer. Chem. Soc.*, 1940, **62**, 2792) reports b. p. 51°, d_4^{20} 1·684, n_D^{20} 1·2685. Fraction (3) appears to be perfluoromethyl*cyclo*hexane which has b. p. 76·2°, d_4^{25} 1·784, n_D^{25} 1·276 (Haszeldine and Smith, *J.*, 1950, 2689).

From the above results it is also evident that the fluorination of 2-methylindole is extremely complicated but that the nitrogen shows some stability to cobalt trifluoride (fractions 5 and 10). Appreciable polymerisation of the fragments takes place (fractions 8—11) and this sometimes involves a nitrogen-containing monomer (fraction 10). Fractions (8)—(10) were viscous oils, and fraction (11) was a glassy solid sublimation of which produced a white powder having a softening point of about 75°.

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55. 1 : 2 : 5 : 6-*Tetrachloronaphthalene.*

By H. A. PIGGOTT and F. H. SLINGER.

BY nitration of 2 : 6-dichloronaphthalene Alén (*Bull. Soc. Chim.*, 1881, **36**, 435) obtained mono-, di-, and tri-nitro-compounds, and the dinitro-compound on fusion with phosphorus pentachloride gave a tetrachloronaphthalene, m. p. 159·5—160·5°. We have confirmed the preparation of the di- and the tri-nitro-compound, although the reaction conditions preferred by us are somewhat different from those recommended by Alén. Reduction of the dinitro-compound to the diamine, followed by a Sandmeyer reaction, gave a tetrachloronaphthalene, m. p. 164°, possibly identical with Alén's tetrachloro-compound.

Among the fifteen tetrachloronaphthalenes prepared by Turner and Wynne (*J.*, 1941, 247) was one of m. p. 164°, designated as a 1 : 2 : 5 : ?-tetrachloronaphthalene since it was obtained from 1 : 2 : 5-trichloronaphthalene by chlorosulphonation followed by fusion with phosphorus pentachloride. A sample of Turner and Wynne's preparation, now in our possession, has been identified with the tetrachloronaphthalene prepared

above, thereby establishing its constitution as 1 : 2 : 5 : 6-tetrachloronaphthalene. Accordingly the dinitro-compound from which we prepared this tetrachloronaphthalene is 2 : 6-dichloro-1 : 5-dinitronaphthalene.

Experimental.—2 : 6-*Dichloro*-1 : 5-*dinitronaphthalene.* 2 : 6-Dichloronaphthalene (12·0 g.) was added gradually to nitric acid (100 c.c.; *d* 1·50) with stirring and ice-cooling to keep the temperature below 20°. After ½ hour's further stirring, the mixture was poured into water (1 l.), and the product filtered off, washed, and dried. The crude product (16·6 g.) was freed from a little trinitro-compound by extraction with hot acetic acid and was crystallised from *o*-dichlorobenzene as needles, m. p. 262—263° (11·5 g.) (Found : N, 9·7; Cl, 24·5. Calc. for $C_{10}H_4O_4N_2Cl_2$: N, 9·8; Cl, 24·8%). Alén gives m. p. 252—253°.

2 : 6-*Dichloro*-1 : 5 : ?-*trinitronaphthalene.* To a solution of 2 : 6-dichloronaphthalene in sulphuric acid (20 c.c.; *d* 1·84), nitric acid (2·6 c.c.; *d* 1·50) was added and the temperature raised to 60° for 18 hours. The mixture was poured into water (200 c.c.), and the precipitated solid filtered off, washed with water and alcohol (to remove a brown by-product), dried, and crystallised from acetic acid. It formed stout prisms, m. p. 204—205° (1·5 g.) (Found : N, 12·7; Cl, 21·4. Calc. for $C_{10}H_2O_6N_3Cl_2$: N, 12·7; Cl, 21·4%) (Alén gives m. p. 198—200°). This compound was also obtained by nitrating 2 : 6-dichloro-1 : 5-dinitronaphthalene under the above conditions.

1 : 5-*Diamino*-2 : 6-*dichloronaphthalene* (plates, m. p. 202—203°, from alcohol) was obtained by reduction of 2 : 6-dichloro-1 : 5-dinitronaphthalene with iron filings and hydrochloric acid in ethylene glycol monoethyl ether (Found : N, 11·85. Calc. for $C_{10}H_8N_2Cl_2$: N, 12·2%). Alén gives m. p. 204—205°.

1 : 2 : 5 : 6-Tetrachloronaphthalene was prepared from 1 : 5-diamino-2 : 6-dichloronaphthalene by a modification of the Hodgson–Walker method (cf. *J.*, 1933, 1620). The diamine (1·0 g.) was dissolved in warm acetic acid (12 c.c.), and sulphuric acid (5 c.c.) was added, giving a fine suspension of the sulphate which was poured slowly into nitrosylsulphuric acid prepared from sodium nitrite (0·8 g.) and sulphuric acid (7 c.c.) at <20°. The resultant viscous liquid was poured into a solution of cuprous chloride (1·1 g.) in concentrated hydrochloric acid (10 c.c.) at 60°. Frothing occurred and, when reaction was complete, water was added to precipitate the product which was then filtered off and crystallised from 2-ethoxyethanol (carbon). White needles (0·6 g.) were obtained, having m. p. 164° alone or mixed with Turner and Wynne's 1 : 2 : 5 : ?-tetrachloronaphthalene (Found : Cl, 53·0. Calc. for $C_{10}H_4Cl_4$: Cl, 53·4%).

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56. *Crystalline* 2 : 3-*Dimethyl* α-D-*Xylose.*

By S. K. CHANDA, E. E. PERCIVAL, and (the late) E. G. V. PERCIVAL.

BY the hydrolysis of methylated esparto xylan Hampton, Haworth, and Hirst (*J.*, 1929, 1739) isolated 2 : 3-dimethyl D-xylose as a syrup, and proved its constitution. This sugar has been isolated several times since (Robertson and Speedie, *J.*, 1934, 824; Chanda, Hirst, Jones, and Percival, *J.*, 1950, 1289; Chanda, Hirst, and Percival, *J.*, 1951, 1240) but invariably in a syrupy condition : it has now crystallised.

Experimental.—Purification of a specimen prepared from methylated esparto xylan (Chanda *et al.*, *loc. cit.*) by passage, in light petroleum (b. p. 100—120°)–*n*-butanol (7 : 3), saturated with water, through a column of powdered cellulose in the usual way, isolation of the product, and repetition of the operation gave a colourless syrup which crystallised completely when kept for 2 years in a vacuum over phosphoric oxide. The crystals had m. p. 79—80°, $[\alpha]_D^{15}$ +70° (3 minutes), +68° (5 minutes), +61° (8 minutes), +48° (20 minutes), +37° (35 minutes), +23° (14 hours, constant) (*c*, 1·0 in water) (Found : C, 47·2; H, 8·1. Calc. for $C_7H_{14}O_5$: C, 47·2; H, 8·9%).

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OBITUARY NOTICES.

ERNEST FOURNEAU.

1872—1949.

ERNEST FRANÇOIS AUGUSTE FOURNEAU was born on October 4th, 1872, at Biarritz, which was also the natal city of the brothers Moureu, with whom he maintained a life-long friendship and by whom he was probably considerably influenced in the choice of a career. On leaving school in 1889 he was apprenticed in the pharmacy of Felix Moureu in Biarritz where he remained for nearly three years. Compulsory military service followed in 1892—1893, after which he proceeded to the École de Pharmacie in Paris, where he had among his fellow students Blaise and Valeur. In 1898 he obtained his diploma as a pharmacien, and then spent a year with Charles Moureu " passing in review most of the methods of organic chemistry." He completed his training with three years of research in Germany under Emil Fischer, Curtius, Gattermann, and finally Willstätter. In those days the production of pharmaceutical chemicals in Britain and France was virtually limited to the few alkaloids and glucosides in use in medicine and Germany was almost the sole source of supply of synthetic drugs. Fourneau returned to France determined to do his utmost to bring his country to the front in this branch of the fine chemical industry. He got the necessary opportunity in the research laboratories of Les Établissements Poulenc Frères, of which he was made Director in 1903 and where he remained until 1911 when Dr. Roux offered him a post as principal of a laboratory of therapeutic chemistry at the Pasteur Institute and in this congenial environment Fourneau passed the remainder of his working life.

While in Germany he published three papers : one with Emil Fischer describing the preparation of molecular anhydrides of amino-acids, such as glycylglycine hydrochloride (*Ber.*, 1901, **34**, 2868), and another with Willstätter on lupinine, in which the simpler empirical formula, $C_{10}H_{19}ON$, was adopted for the alkaloid and a beginning was made in the determination of its structure, which it was suggested consisted of a bicyclic system (*ibid.*, 1902, **35**, 1910) similar to that postulated for cinchonine, then under investigation by Königs and others; the third paper was by Fourneau alone on 9-phenyladenine, prepared by the general method of treating the appropriate trichloropurine with ammonia and reducing with hydriodic acid the resulting 6-amino-2 : 8-dichloro-9-phenylpurine (*ibid.*, 1901, **34**, 112).

In attempting an appreciation of Fourneau's work it must be remembered that he had a remarkable flair for envisaging the kind of molecular structure which would produce a particular pharmacological effect, that for him chemistry was applicable to a wide range of therapeutics, and that he was gifted with a notable capacity for taking pains to bring his ideas to practical fruition.

According to his own statement (*J. Pharm. Chim.*, 1910, [vii], **2**, 56) his work on amino-alcohols and their derivatives with therapeutic properties arose from the consideration that the two natural local anæsthetics, cocaine and tropacocaine, are *o*-benzoyl derivatives of the methyl ester of an amino-hydroxy-acid (ecgonine) and of an amino-alcohol (*pseudo*tropine) respectively. He thought it might be possible to accommodate these functions on a simpler nucleus to produce a substance having at least qualitatively a similar pharmacological effect. He also suggested that a simple nucleus might be devised on which a series of derivatives could be constructed with various therapeutic properties, *e.g.*, hypnotics, sedatives, antipyretics, etc.

The first series of amino-alcohols, represented by the general formula, $HO{\cdot}CMeR{\cdot}CH_2{\cdot}NMe_2$, prepared by Fourneau (*Compt. rend.*, 1904, **138**, 766) included six members in which R was represented by Me, Et, Pr^n, *iso*amyl, Ph, and $Ph{\cdot}CH_2$. In each case the benzoyl derivative formed a hydrochloride having local anæsthetic action. In the best of the series R was ethyl, and this compound on benzoylation and conversion into the hydrochloride became the well-known local anæsthetic stovaine, $Ph{\cdot}CO{\cdot}O{\cdot}CMeEt{\cdot}CH_2{\cdot}NMe_2,HCl$. Later, with Ribas (*Anal. Fís. Quím.*, 1927, **25**, 401) the amino-alcohol (stovaine base) was resolved into (+)- and (−)-forms and these were converted into (+)- and (−)-stovaines of which the former was the more potent local anæsthetic. Stovaine base was also condensed with anhydrous chloral, producing the semi-acetal $Me_2N{\cdot}CH_2{\cdot}CMeEt{\cdot}O{\cdot}CH(OH){\cdot}CCl_3$, which as the benzoate hydrochloride proved to be a potent local anæsthetic, although its salts were acid and too irritant for practical use (with Mlle. Brydona, *Bull. Soc. chim.*, 1928, [iv], **43**, 1023). An intensely active local anæsthetic was also obtained when benzoyl was replaced by *p*-aminobenzoyl in stovaine, as was done in preparing a series of homologues of novocaine $Et_2N{\cdot}CH_2{\cdot}CH_2{\cdot}O{\cdot}CO{\cdot}C_6H_4{\cdot}NH_2$ (with Puyal, *ibid.*, 1922, [iv], **31**, 424).

The second idea, that the pharmacological action might be varied by change in substituents, was also realised to some extent in this series, *e.g.*, the valeryl esters of the amino-alcohols were sedative and some of the esters with aromatic acids were antipyretics (*J. Pharm. Chim.*, 1910, [vii], **2**, 57) but these activities were either not well-developed or the derivative was toxic.

A later series (*ibid.*, pp. 337, 397) provided further evidence; it included derivatives of the simple aminomethylethylmethylcarbinol, $HO{\cdot}CMeEt{\cdot}CH_2{\cdot}NH_2$, corresponding to the stovaine base. This gave with valeryl chloride an amide $HO{\cdot}CMeEt{\cdot}CH_2{\cdot}NH{\cdot}CO{\cdot}CH_2{\cdot}CHMe_2$, with ethyl chloroformate a urethane $HO{\cdot}CMeEt{\cdot}CH_2{\cdot}NH{\cdot}CO_2Et$, and with propyl chloroformate the corresponding propyl ester. With potassium cyanate the same alcohol furnished the substituted urea $HO{\cdot}CMeEt{\cdot}CH_2{\cdot}NH{\cdot}CO{\cdot}NH_2$. The urethanes were hypnotic in action when given in large doses, *e.g.*, 0·4 g. per kg. of body weight in rabbits. It was suggested that their lower potency, compared with that of the similarly constituted amylurea

$$CH_3{\cdot}CH_2{\cdot}CMe_2{\cdot}NH{\cdot}CO{\cdot}NH_2,$$

is a result of their greater solubility in water owing to the presence of the alcoholic hydroxyl group.

At first, these amino-alcohols were obtained by treating with ammonia or amines the appropriate chlorohydrins prepared by Tiffeneau's method, the action of alkylmagnesium halides on chloro-ketones (*Compt. rend.*, 1902, **134**, 774), but later other methods were added; stovaine base, for example, was also made by the action of ethylmagnesium bromide on dimethylaminoacetone or by treating 2-ethyl-2-methylethylene oxide with dimethylamine (*J. Pharm. Chim.*, 1910, [vii], **2**, 109).

Ethylene oxides first became available with the preparation of β-methylstyrene oxide in 1905 (Klages, *Ber.*, 1905, **38**, 1969; Tiffeneau, *Compt. rend.*, 1905, **140**, 1458), and the study of their preparation, constitution, and reactions was continued by Fourneau and Tiffeneau and later by Fourneau and Ribas in a series of papers published from 1905 to 1927. When phenol is heated with epichlorohydrin in a closed tube, at least four products are formed, two of which are interconvertible, *viz.*, the oxide, phenylglycide ether $PhO{\cdot}CH_2{\cdot}CH\langle^{CH_2}_{O}$ and the chlorohydrin $PhO{\cdot}CH_2{\cdot}CH(OH){\cdot}CH_2Cl$. Similar glycide ethers from *p*-cresol and α-naphthol were described by Lindemann (*Ber.*, 1891, **24**, 2145). They all react with amines to form compounds of the type 3-dimethylamino-1-phenoxypropan-2-ol $PhO{\cdot}CH_2{\cdot}CH(OH){\cdot}CH_2{\cdot}NMe_2$, and Fourneau prepared a series (*J. Pharm. Chim.*, 1910, [vii], **1**, 55, 97) in which the phenoxy-group was replaced by *p*-tolyloxy, guaiacyl, thymyloxy, etc. All of them had antipyretic and analgesic properties but owing to their cardiac action they proved unsuitable for therapeutic use. This work was later (with Billeter and Bovet, *ibid.*, 1934, [viii], **19**, 49) extended to glycidic esters $CRR'{\cdot}\underset{\llcorner O \lrcorner}{CH}{\cdot}CO_2Et$, which with ammonia or primary amines in aqueous alcohol gave quantitative yields of the corresponding amides $CRR'{\cdot}\underset{\llcorner O \lrcorner}{CH}{\cdot}CO{\cdot}NR''R'''$. These, in experiments on rabbits, mice, and fish, showed a rapid narcotic action of short duration. The study was continued (with Billeter, *Bull. Soc. chim.*, 1940, [v], **7**, 593) as a detailed examination of the more complex action of ammonia, aliphatic amines, or arylamines on phenylglycidic esters.

Fourneau also prepared several series of amino-alcohols to ascertain the effect of changes in structure on pharmacological action, *e.g.*, the group of αγ-amino-alcohols, such as 1-dimethylaminohexan-3-ol, obtained by the action of dimethylamine on the chlorohydrins resulting from the interaction of chloropropaldehyde and the appropriate alkylmagnesium bromides. The hydrochlorides of the benzoates of these secondary alcohols showed anæsthetic action less durable than that of stovaine (with Mme. Ramart-Lucas, *ibid.*, 1919, [iv], **25**, 363; 1920, [iv], **27**, 386, 550).

Most of the amino-alcohols so far mentioned contain either a secondary or a tertiary alcohol group, and in 1930 in association with Mlle. Benoit and Firmenich (*ibid.*, 1930, [iv], **47**, 858) the investigation of this type of variation was completed with a series containing primary alcohol groups obtained by the action of methyl- or dimethyl-amine on the bromoacetins $R^1R^2C(CH_2Br){\cdot}CH_2{\cdot}O{\cdot}CO{\cdot}CH_3$ resulting from the action of hydrobromic acid on diprimary glycols in acetic acid. The benzoates of these compounds were local anæsthetics but seemed to present no practical advantage over cocaine or stovaine with which they were compared.

The ephedrines, which Fourneau regarded as amino-alcohols rather than as alkaloids, occupied his attention at intervals from 1904 to 1945. Of the nine papers published on this subject several describe syntheses of ephedrines, and one (with Kanao, *ibid.*, 1924, [iv], **35**, 614) is of general interest as it included a useful bibliography of this subject up to that date, described

and discussed the reactions used in the various syntheses of the ephedrines, cleared up possible confusion between ephedrine and the *pseudo*- and *iso*-forms, and indicated which was formed in the various syntheses recorded. Later, with Barrelet (*Anal. Fís. Quím.*, 1929, **27**, 500) he synthesised three homologues of ephedrine and in 1945 (with Mlle. Benoit, *Bull. Soc. chim.*, 1945, [v], **12**, 985) described the interesting results of a study of the action of methylamine on the mixture of *cis*- and *trans*-forms of β-methylstyrene oxide formed when β-methylstyrene is treated with benzoyl peroxide. *iso*Ephedrine is usually the predominant product; ephedrine and *pseudo*ephedrine are also formed, but little or no *iso*-ψ-ephedrine.

Among other types of local anæsthetics may be mentioned a series derived from piperazine and made by the interaction of piperazine hydrate and ethylene oxides—for example, 1-(2-hydroxy-2-methyl-*n*-butyl)piperazine $HO{\cdot}CMeEt{\cdot}CH_2{\cdot}N<[CH_2]_4>NH$ and the disubstituted bis-compound $HO{\cdot}CMeEt{\cdot}CH_2{\cdot}N<[CH_2]_4>N{\cdot}CH_2{\cdot}CMeEt{\cdot}OH$, both made from ethylmethylethylene oxide (with Barrelet, *ibid.*, 1929, [iv], **45**, 1172) and of which a higher homologous series was made (with Samdahl, *ibid.*, 1930, [iv], **47**, 1003). These substances from the butyl derivatives upwards were definitely local anæsthetics but some of them proved irritant to the mucous membrane and the cornea. Finally it was found that phenanthrols are converted by 2-diethylaminoethyl chloride into amino-ether oxides (with Matti, *ibid.*, 1940, [v], **7**, 615; 1942, [v], **9**, 633), of which the 1-, 2-, 3-, 4-, and 9-2′-diethylaminoethoxyphenanthrenes were prepared and also the 9 : 10-disubstituted product. The mono-series proved to be local anæsthetics but none was analgesic; the disubstituted substance produced intense anæsthesia when applied to the tongue. The more interesting observation was also recorded that while the monosubstituted products were cardiac sedatives and lowered excitability of the myocardium, the single disubstituted product obtained, viz., 9 : 10-bis(diethylaminoethoxy)phenanthrene dihydrochloride, was very toxic.

Of the two bases, ψ-tropine and ecgonine, which Fourneau had in mind as models when the work on local anæsthetics was started, all products so far described are based on the ψ-tropine or amino-alcohol type. Work on the ecgonine or amino-hydroxy-acid type was much less fruitful. The first acid used was phenyl-lactic acid (*ibid.*, 1907, [iv], **1**, 549); "the benzoyl derivatives of ethyl phenyldimethylaminolactate $Me_2N{\cdot}CH(CO_2Et){\cdot}CHPh{\cdot}O{\cdot}COPh$" was an effective local anæsthetic but too acid for practical use. Attention was then given to hydroxy-*iso*butyric acid: the propyl ester of the derived chloro-derivative was converted into propyl β-dimethylamino-α-hydroxy*iso*butyrate of which the valerate $Me_2N{\cdot}CH_2{\cdot}CMe(CO{\cdot}OC_3H_7){\cdot}O{\cdot}CO{\cdot}C_4H_9$ as the hydrobromide had some success as a hypnotic under the name "Quietol" although the benzoyl derivative of the ethyl ester, which had intense anæsthetic properties when applied to the tongue, like the previous compound, was too acid for use (*J. Pharm. Chim.*, 1908, [vi], **27**, 513; *Bull. Soc. chim.*, 1909, [iv], **5**, 229); and the insertion of a second dimethylamino-group failed to overcome this difficulty (*ibid.*, 1921, [iv], **29**, 413).

Apart from the hypnotics arising out of the work described above, Fourneau also investigated certain other series providing such drugs, notably the ureides of bromovaleric acids on which three comprehensive papers were published (with Florence, *ibid.*, 1927, [iv], **41**, 1518; 1928, [iv], **43**, 211, 1027), dealing especially with the interrelations of chemical structure and hypnotic action. It was found in a comparative study using the known α-bromo*iso*valerylurea, $CHMe_2{\cdot}CHBr{\cdot}CO{\cdot}NH{\cdot}CO{\cdot}NH_2$, and α-bromo-α-ethylbutyrylurea, $CEt_2Br{\cdot}CO{\cdot}NH{\cdot}CO{\cdot}NH_2$, as reference standards, that the effect of displacement of the bromine atom in the chain, and accumulation of bromine atoms, confirmed the relation already observed by Overton between the partition coefficient in oil and water and the hypnotic properties, and supported Tiffeneau's statement that the relation holds only within the same series. Migration of the bromine atom from the α- to the β-position reduced the hypnotic action, the partition coefficient, and the solubility in water, but it was concluded later that the α-position of the bromine atom is not specific, active ureides with the bromine in β- and γ-positions being also found. Branching in the chain produced a marked increase in solubility in water and in the partition coefficient, and in this series the partition coefficient and hypnotic action were closely parallel.

Among other series of pharmacologically active compounds prepared and investigated by Fourneau, were derivatives of benzodioxan and aminocoumarans, which simulate the sympathicolytic action of yohimbine and ergotamine (with Maderni and Mme. de Lestrange, *J. Pharm. Chim.*, 1933, [viii], **18**, 185), of which the most interesting appeared to be 3-diethylaminomethylbenzodioxan which suppresses and reverses the vasoconstrictive action of adrenaline and induces lowering of body temperature in rabbits (with Bovet, *Compt. rend. Soc. Biol.*, 1933, **113**, 388). Attention was also directed to the intense acetylcholine type of activity shown in a series of amino-acetals of polyhydric alcohols, which is at its peak in 2 : 3-ethylidenedioxy-

propyltrimethylammonium iodide (I; R = H, R′ = Me) (with D. Bovet, F. Bovet, and Montézin, *Bull. Soc. Chim. biol.*, 1944, **26**, 516). This line of work was then extended to the examination of muscarine-like action in amino-acetals of the general types (I) and (II). All the examples examined showed this type of action, which reached a maximum in type (I) with R = H and R′ = Me, and in type (II) with R = H, but fell off when R or R′ was increased and disappeared when R = R′ = Ph. It was also reduced by the insertion of a hydroxyl group (with Bovet, Montézin, J. P. Fourneau, and Mlle. Chantalou, *Ann. Pharm. Franç*, 1944, **2**, 120; 1945, **3**, 114). This was followed by a paper dealing with the complex reactions, simulating those of nicotine, muscarine, and curare, shown by a series of halogenated derivatives of alkyltrimethylammonium salts (with D. Bovet and F. Bovet, *ibid.*, 1946, **4**, 166).

(I) $I\{Me_3N\cdot CH_2\cdot CH(O\cdot CRR'\cdot O\cdot CH_2)$ (II) $I\{Me_3N\cdot CH_2\cdot CH(O\cdot CHR\cdot CH_2)O$

The work so far mentioned relates almost wholly to symptomatic drugs, but Fourneau also made notable contributions to chemotherapy proper. In the early years of this century great interest was aroused by Thomas's successful treatment of trypanosomiasis in animals with atoxyl (*Proc. Roy. Soc.*, 1905, *B*, **76**, 589) which was shown by Ehrlich and Bertheim to be sodium hydrogen *p*-aminophenylarsonate, $p\text{-}NH_2\cdot C_6H_4\cdot AsO(OH)\cdot ONa$ (*Ber.*, 1907, **40**, 3292), and they in common with Fourneau (*J. Pharm. Chim.*, 1907, [vi], **25**, 332, 528) agreed that it was first made by Béchamp in 1863. This inaugurated a period of great activity, during which the important drug " Salvarsan " was discovered in Germany, and Ehrlich and Hata published their conclusions on the therapeutic superiority of organic arsenical compounds containing arsenic in the tervalent form over the phenylarsonic acid series in which arsenic is quinquevalent (Die experimentelle Chemotherapie der Spirillosen, Berlin, 1911). Fourneau gave reasons for disagreement with these views and promised further work on the subject (*Ann. Inst. Pasteur*, 1921, **35**, 571). The results were published in two remarkable papers (*ibid.*, 1923, **37**, 551; 1926, **40**, 933) in which he had as colleagues M. Navarro-Martin, M. and Mme. Tréfouel, and Mme. de Lestrange-Trévise. The preparation of a large number of arsenical compounds, many of them already known, was described and there was recorded for each the maximum tolerated dose and the effective curative dose in animals infected with trypanosomes or spirochætes. The effects, on efficiency against each type of infection, of the nature and orientation of each substituent alone or in conjunction were discussed in detail. Among the points stressed by Ehrlich and Hata against quinquevalent arsenic compounds was their liability to cause nervous disorders including ocular trouble, and Fourneau was at pains to point out that as a number of his compounds did not produce these effects in the experimental animals this kind of action could not be attributed to the arsonic acid group. Among the compounds dealt with were the ten isomeric aminohydroxyphenylarsonic acids, which were fully described later, with a comparison of their pharmacological constants (with M. and Mme. Tréfouel and Mlle. Benoit, *Bull. Soc. chim.*, 1927, [iv], **41**, 499), and one of these in the form of its *N*-acetyl derivative, *viz.*, 3-acetamido-4-hydroxyphenylarsonic acid, is the well-known drug " Stovarsol " which was adopted in the British Pharmacopœia in 1936 under the name acetarsol. The drug now seems to be used principally for the treatment of amœbiasis and *Trichomonas vaginalis* and for congenital syphilis in young children because it can be administered orally.

It should be noted that 3-amino-4-hydroxyphenylarsonic acid had already been made by Ehrlich and Bertheim (*Ber.*, 1912, **45**, 756) during their preparation of " Salvarsan," but its recognition as the basis of a useful drug was undoubtedly the result of the careful work of Fourneau and his colleagues on its preparation and pharmacological action. The series also included " Orsanine," 4-acetamido-2-hydroxyphenylarsonic acid, which was rather more toxic than " Stovarsol " and unlike the latter is an effective trypanocide (cf. Walls, *Chem. and Ind.*, 1951, 607).

Among other arsenic compounds prepared by Fourneau may be mentioned the optically active forms of methyltryparsamide (*N*-*p*-arsonophenyl-β-methylglycine amide,

$$NH_2\cdot CO\cdot CHMe\cdot NH\cdot C_6H_4\cdot AsO_3H_2),$$

obtained by the use of quinine, which were used for the resolution of synthetic (±)-ephedrine (with Nicolitch, *Bull. Soc. chim.*, 1928, [iv], **43**, 1232).

Special interest also attaches to the series of diarsonic acid derivatives of various aryl nuclei, which exhibited a reversal of the trypanocidal action of the monoarsonic acids, being active against *T. congolense* but inactive towards *T. brucei* (with M. and Mme. Tréfouel, Bovet, and

Koetschet, *Compt. rend.*, 1933, **196**, 1173) and to the so-called " alcoholic arsonic acids," such as 4-amino-3-hydroxymethylphenylarsonic acid, $NH_2 \cdot C_6H_3(CH_2 \cdot OH) \cdot AsO_3H_2$, which in pharmacological tests in mice proved 3—4 times as active as atoxyl, indicating that the $CH_2 \cdot OH$ group had a favourable influence on trypanocidal action (with Mme. de Lestrange, *Bull. Soc. chim.*, 1933, [iv], **53**, 330).

Fourneau also published five papers on organic mercury compounds of which two with K. I. Melville (*J. Pharm. exp. Ther.*, 1931, **41**, 21, 47) deal with mercurial chemotherapy, the first being a study of the evaluation and mechanism of mercurial toxicity in rabbits and the correlation of the various types of this action with chemical structure in the four groups of compounds tested. In the second paper a method is developed for the quantitative determination of the diuretic activity of mercurial compounds by intravenous injection into rabbits, the results being expressed in the form of a chemotherapeutic coefficient. All the soluble mercury compounds tested were active, but no well-defined relation between degree of activity and chemical structure was found.

Fourneau and his colleagues, M. and Mme. Tréfouel, also made important contributions to the chemotherapy of malaria. They first examined the biological method of estimating the efficacy of an antimalarial drug, involving the use of canaries experimentally infected with avian malaria (*Plasmodium relictum*), and, having developed a standard method (with Stefanopoulo, Mlle. Benoit, Mme. de Lestrange, and K. Melville, *Ann. Inst. Pasteur*, 1930, **44**, 503), tried it out on a few known antimalarial drugs including plasmoquin, which had become available in 1926, and then proceeded to apply the test to well over 100 substances of varied types, including complex amino-alcohols, several kinds of quinoline derivatives, and organic compounds of antimony, arsenic, lead, and mercury. The preparation of these compounds was described separately (with Mlle. Benoit, *ibid.*, p. 719; with Wancolle, *Bull. Soc. chim.*, 1930, [iv], **47**, 738). This investigation may be regarded as a preliminary screening test and it afforded no positive results of importance. In two further papers (with Bovet and Mlle. Benoit, *Ann. Inst. Pasteur*, 1931, **46**, 514; 1933, **50**, 731) a series of substituted quinolines of the plasmoquin type were used in biological tests on the Java sparrow or rice finch (*Orizonis orizivora*), which always carries a natural infection of *Hæmoproteus orizivoræ* similar in type to the malaria parasite. The substances used were mainly 6-alkoxy-8-aminoquinolines derivable from the general formula $RO \cdot Q \cdot NH \cdot [CH_2]_n \cdot NR'_2$. $[CH_2]_n$ was also in many cases changed to a branched chain or a branched oxygenated chain, either of which had a dystherapeutic effect. Among a number of promising compounds final choice was made of 8-3′-diethylaminopropylamino-6-methoxyquinoline which had a chemotherapeutic index of 1 : 100 compared with 1 : 40 recorded for plasmoquin (side-chain $\cdot NH \cdot CHMe \cdot [CH_2]_3 \cdot NEt_2$) in the same series of tests. This substance, first distinguished as F710, was subsequently named " Rhodoquine " in France and later " Plasmocide " in Russia.

Two other drugs of German origin also attracted Fourneau's attention. In 1921 biological and clinical reports began to appear about a new and effective trypanocide, Bayer 205, the chemical structure of which was not disclosed. It aroused intense curiosity and was investigated by Fourneau and his colleagues, who assigned to the drug the now well-known formula and described the preparation of F309, which was soon generally accepted as identical with Bayer 205 (with M. and Mme. Tréfouel and Vallée, *Compt. rend.*, 1924, **178**, 675). A full account of the drug including a review of the already extensive literature was given as an introduction to a description of their own researches on ureas of aminobenzamidonaphthalenesulphonic acids, for which the results of biological tests with comments on the variation of these with changes in chemical structure were recorded, the most interesting feature being in some cases the large effect on trypanocidal action induced by a small deviation in structure (*Ann. Inst. Pasteur*, 1924, **38**, 81). The second drug was " Prontosil," 2 : 4-diamino-4′-sulphamylazobenzene hydrochloride, to which attention was first called by Domagk's paper (*Deut. med. Woch.*, 1935, **61**, 250) describing its action on mice infected with hæmolytic streptococci. After it had been shown (M. and Mme. Tréfouel, Nitti, and Bovet, *Compt. rend. Soc. Biol.*, 1935, **120**, 756) that the antibacterial action of this drug was probably due to its breakdown in the body into sulphanilamide, which they found to be active against streptococci in the mouse and rabbit (cf. Buttle, Gray, and Stephenson, *Lancet*, 1936, **230**, 1286), considerable interest was aroused in the investigation of the range of activity of sulphanilamide and its analogues and derivatives, to which Fourneau and his group made two important contributions (*Compt. rend. Soc. Biol.*, 1936, **122**, 258, 652). In the first they dealt with the action of 130 compounds, all related to or analogous with sulphanilamide, and showed that changes in the character, or orientation of the two substituents, or addition of a third, usually reduced or even abolished streptococcidal

action, which however was retained after diazotisation and coupling. The second paper dealt with the inhibition of moulds which was found to run parallel with the action on streptococci.

The same group of workers then began work on other sulphur compounds (*Compt. rend.*, 1937, **204**, 1763; **205**, 299) and recorded that in the 4 : 4′-dinitrodiphenyl series the sulphide and disulphide were active but inferior to sulphanilamide, whereas the sulphone was more active than the latter against streptococcal infection in mice. It was also active, in higher doses, against pneumococcal septicæmia. About the same time the dinitro- and the diamino-sulphones were under examination in England by Buttle, Stephenson, Smith, Dewing, and Foster (*Lancet*, 1937, **232**, 1331). Their results were in general agreement with the French findings for the dinitro-compound, but they were much more concerned with the more toxic diamino-derivative, which proved to be 100 times as active as sulphanilamide against streptococcal infections and was also effective in prolonging the lives of mice infected with pneumococcus. The obstacle to therapeutic use of this new and potent antibacterial agent was its toxicity and this was overcome when the French group investigated the diacetyl derivative, di-(*p*-acetamidophenyl) sulphone, which they found to be 10 times as active as sulphanilamide against either streptococcal or pneumococcal infections in mice; it was also much less toxic than the parent diamino-compound.

As every chemist knows, there have been enormous developments from these early observations and to-day there is almost a special section of therapeutics based on what are colloquially called " sulpha " and " sulphone " drugs.

In addition to the work on ephedrine already referred to, Fourneau was concerned with two other alkaloids, Hesse's quebrachine, which was identified as yohimbine (with Page, *Bull. Sci. Pharmacol.*, 1914, **21**, 7), and corynanthine; the latter he isolated from *Pseudocinchona africana*, and showed that it was isomeric with yohimbine (*Compt. rend.*, 1909, **148**, 1770; with Fiore, *Bull. Soc. chim.*, 1911, [iv], **9**, 1037) and yielded on acid hydrolysis corynanthic acid. On alkaline hydrolysis an acid of lower rotation was produced, which on methylation did not reproduce corynanthine, but according to some authorities a mixture of corynanthine and yohimbine (*Compt. rend.*, 1910, **150**, 976; with Mlle. Benoit, *Bull. Soc. chim.*, 1945, [v], **12**, 934).

The last paper Fourneau published was written with Professor Janot and dealt with the chemistry of the curares, the *Erythrina* alkaloids, and synthetic substances exhibiting a curare-like action (*Ann. Pharm. Franç.*, 1948, **6**, 406; 1949, **7**, 353), and to add to the value and exhaustive character of this useful review it was accompanied by a historical and botanical section contributed by Paris (*ibid.*, 1949, **7**, 346) and a chapter on the standardisation of curares by Cheymol and Mlle. Corteggiani (*ibid.*, p. 368). It included an account of the action of " Flaxedil," 1 : 2 : 3-$C_6H_3(O{\cdot}CH_2{\cdot}CH_2{\cdot}NEt_3I)_3$, one of a series of phenolic ethers of quaternary ammonium bases possessing curare-like properties, described by Mme. de Lestrange (*ibid.*, 1948, **6**, 450) and examined pharmacologically (Bovet, Depierre, and Mme. de Lestrange, *Compt. rend.*, 1947, **225**, 74) with results promising enough to warrant therapeutic trials in France and in England (see, for example, Mushin, Wien, Mason, and Langston, *Lancet*, 1949, **256**, 726; Doughty, *ibid.*, 1950, **258**, 899).

There are a number of other investigations to which limitation of space precludes reference, but the foregoing account is probably sufficient to indicate the wide range of Fourneau's interests and his unique capacity for dealing with chemotherapeutic problems. In spite of his preoccupation with experimental work he found time to write numerous reviews on special drugs, such as the two on organic arsenical compounds and curare already referred to, and he frequently contributed reports on the current prospects of and developments in chemotherapy to special Congresses, such as that on " The Relations between the Chemical Constitution of Compounds and their Therapeutic Action " submitted to the 6th International Chemical Congress held at Bucarest in 1925. In the bibliography appended to his admirable " Notice sur la Vie et les Travaux de Ernest Fourneau " (*Bull. Soc. chim.*, 1950, 953) Professor Delépine has published a list of thirty such reviews contributed by Fourneau from 1902 to 1949. Fourneau was also much concerned for the progress of the pharmaceutical industry, especially in France, as evidenced by his writings on patents and on the organisation of research in chemotherapy.

He did not lack appreciation of his work during his lifetime, for he received many honours both in France and abroad, and the memoirs published since his decease on the 5th August, 1949, bear witness not only to great achievements in his chosen subject but also to the personal inspiration, which attracted and retained for so long a group of devoted colleagues and disciples.

T. A. Henry.

ALBERT EDWARD GILLAM.

1900—1950.

THE death of Dr. A. E. Gillam occurred, after a long illness, at his home in Timperley, Cheshire, on January 16th, 1950.

Although never having received an academic training in the usual sense, he rose, by dint of exceptional endeavour and great enthusiasm, from being a laboratory assistant in Liverpool to the position of senior lecturer at Manchester University at the time of his death. Gillam was a pioneer in the use of ultra-violet absorption spectra for the solution of analytical and structural problems in organic chemistry, and his contributions have assisted materially in paving the way for the truly remarkable use made of these methods today.

Gillam joined the laboratory staff of the Department of Inorganic Chemistry in the University of Liverpool, then under the direction of Professor E. C. C. Baly, immediately after leaving school. He eventually became principal lecture demonstrator and in the evenings he studied at the Central Technical School, obtaining the Associateship of the Royal Institute of Chemistry in 1926, and subsequently becoming a part-time assistant lecturer at the College. Alongside his work as lecture demonstrator he began research under the direction of Professor R. A. Morton and studied the absorption spectra of halogens and interhalogen compounds (*Proc. Roy. Soc.*, 1929, *A*, **124**, 604), methods of determining ultra-violet light intensity (*J. Soc. Chem. Ind.*, 1927, **46**, 417), and other related topics. He was awarded the M.Sc. degree in 1929 for an outstanding thesis on this work.

In due course, under Professor Sir Ian Heilbron and Professor R. A. Morton, Gillam became associated with those pioneering studies in the application of ultra-violet and visual light absorption measurements to the chemistry of natural products, an application which in the past two decades has proved so fruitful. He played an important part in the development of the now well-known methods of estimating vitamin A and carotene (*Biochem. J.*, 1931, **25**, 30, 1346, 1352), introducing a modified method of determining the butter-fat content of these substances (*Biochem. J.*, 1934, **28**, 79). This work led directly to one of Gillam's most significant contributions to organic chemistry. In Manchester with El Ridi in 1935, he made the pioneer observation of the occurrence of geometrical isomerism in the series of C_{40} carotenoids, an observation which was followed by the extensive fundamental development of this subject in the hands of Zechmeister and his school. During an examination of the carotenoids present in butter unsaponifiable matter, the extracted pigments were fractionated by adsorption on alumina and examined spectroscopically, and at first it appeared that the β-carotene present was accompanied by appreciable quantities of α-carotene (*Biochem. J.*, 1936, **30**, 1735). Separate experiments with the pure β-isomer then led to the conclusion that β-carotene underwent a reversible change into another pigment on adsorption on alumina. Subsequently the new pigment was isolated in a pure condition by repeated chromatography, and it was named *pseudo*-α-carotene, because of its spectroscopic similarity to the α-isomer. The isomerisation of α-carotene was also studied (*Biochem. J.*, 1937, **31**, 1605) and another new pigment, *neo*-α-carotene, was isolated and characterised. It was initially concluded that the change apparently brought about by the adsorption process involved either the migration of a double bond out of conjugation or else geometrical isomerism, the latter explanation subsequently being preferred after the discovery that isomerisation of α-carotene resulted in little change in the growth-promoting activity. Gillam's work in this field was closely followed by that of Zechmeister and Tuzson (*Biochem. J.*, 1938, **32**, 1305) who, finding that lycopene and cryptoxanthin isomerised spontaneously in solution, suggested that such was also the case with the carotenes, the adsorption procedure serving only to separate the preformed mixture of isomers. This suggestion was fully confirmed by Carter and Gillam (*Biochem. J.*, 1939, **33**, 1325), an equilibrium mixture of β-carotene (70%) and *pseudo*-α-carotene (30%) being found to be produced when the former was heated in benzene at 80° for three hours. Some idea of the extent of the ultimate development of this work can be gleaned from the review by Zechmeister (*Chem. Reviews*, 1944, **34**, 267).

In 1940 Gillam published the first paper (with Booker and Evans, *J.*, 1940, 1453) in a series in which it was " planned to collect experimental data on the absorption spectra of compounds containing specific light-absorbing groups of atoms with the object of discovering the effect of various well-defined molecular environments on the resulting absorption spectra." It was pointed out for the first time that the absorption spectra of conjugated dienes were affected by the degree of substitution of the chromophoric system. Thus hexa-2 : 4-diene absorbs

maximally at 2270 Å, the two terminal methyl groups being responsible for the shift of 100 Å from the position of absorption of the parent butadiene. A similar state of affairs was found to exist in the αβ-unsaturated ketones (Evans and Gillam, *J.*, 1941, 815). These studies, simultaneous with the more extensive treatment by Woodward (*J. Amer. Chem. Soc.*, 1941, **63**, 1123; 1942, **64**, 72) and the further extension by Fieser and Fieser (cf. " Natural Products Related to Phenanthrene," 1949, p. 184) to the steroid series, whereby widely applicable rules have been enunciated relating position of maximal absorption to degree of substitution, have materially assisted the organic chemist in tackling structural problems.

Gillam first investigated absorption spectra of terpenoid compounds in association with Professor Sir John Simonsen, it being demonstrated that the sesquiterpene ketone, α-cyperone, like eremophilone, was αβ-unsaturated (*J.*, 1936, 676). With T. F. West, the investigation, by spectrographic methods, of a number of hitherto obscure structural problems was later undertaken. These studies included the elucidation of the nature of the chromophoric system in irone (*J.*, 1942, 95) and the observation and discussion of the peculiar light-absorption properties of umbellulone (*J.*, 1945, 95). As a result of a study of isothujone (*J.*, 1941, 811) the distinctly anomalous absorption properties of unsaturated ketones containing a five-membered ring were noted, the maxima being at about 100 Å to shorter wave-lengths than the calculated values, one of the few instances where the Woodward rules do not apply.

Gillam and West made an important contribution to the determination of the structure of the pyrethrins, the potent insecticidal constituents of pyrethrum flowers (*J.*, 1942, 71; 1944, 49). They demonstrated, in a particularly elegant manner, that the light-absorption properties of the ketonic hydrolysis product, pyrethrolone, resulted from the presence in the molecule of a side-chain conjugated-diene chromophore in addition to an αβ-unsaturated ketone system contained in a five-membered ring. These two systems give rise to additive absorption in the 2280-Å region but, by use of subtraction methods and the light-absorption data for the ketone and the tetrahydro-ketone and their respective semicarbazones, conclusive evidence of the presence of a disubstituted conjugated-diene system, absorbing at about 2260 Å was obtained.

Gillam came with Heilbron to Manchester as a research assistant in 1933 and then was appointed special lecturer in 1935. He was awarded the D.Sc. degree of Liverpool University in 1937 and promoted to senior lecturer in 1946. In Manchester Gillam was responsible for the supervision of all the routine spectrographic work of the rapidly growing research department, an activity which afforded him much scope and the opportunity of increasing greatly the range of substances examined. In addition to work on the polyenes, including vitamin A and the carotenoids, he encountered steroids, triterpenes, and synthetic compounds of many types, and his able and willing collaboration is acknowledged in many of the papers published from Manchester during this period. His assistance was indeed willingly given and he devoted much time, which he could doubtless have well used in pursuing his own problems, to helping research students and his colleagues with theirs. Always the careful investigator, fully conscious of the limitations of the technique he employed, Gillam could never be persuaded to allow his results to support conclusions which he did not feel were amply justified, and wishful thinking on the part of his associates was kindly but firmly discouraged.

For many years Gillam was responsible for the teaching of chemistry to Manchester medical students, an arduous and often unrewarding task which he discharged in a characteristically efficient manner. He was a meticulous teacher, remarkably patient and sympathetic. He served as local representative of the Chemical Society for six years, and for the Bureau of Abstracts he undertook in 1946 the onerous task of compiling the new formula index. His interest in nature study was deep and his knowledge extensive, particularly in ornithology.

Gillam will long be remembered in Manchester as a loyal, helpful, and unfailingly courteous colleague. His courage and cheerfulness made a lasting impression on those who saw him in his last few months. He left a widow and two sons, the elder of whom will soon be going to the University to study chemistry.

E. R. H. Jones.

HEINZ PETER KOCH.

1918—1951.

THE tragic death of Peter Koch in a mountaineering accident on Snowdon on March 24th has removed from our midst a young scientist who, by virtue of his ability as an organic chemist and his wide experience of the application of spectrographic methods, seemed destined to make valuable contributions to the advancement of chemistry.

Koch was born in Germany in 1918 and he came to this country with his parents in 1935. After two years at St. Paul's School he gained an Entrance Scholarship to Imperial College of Science and Technology where he graduated with First Class Honours in 1939, being awarded the Hofmann Prize for Organic Chemistry. He began research under the direction of Professor Sir Ian Heilbron and one of the writers in 1939 and, in spite of considerable interruption and the necessity, common in those days, of changing his subject owing to the exigencies of war-time requirements, he completed a thesis and obtained the degree of Ph.D. in 1942 (see *J.*, 1942, 393, 735). His interest in spectrography began at that time in a desire to explain some of the anomalous light-absorption results which were noted during the work. Arising out of studies on acetylmethyl*cyclo*hexene, Koch discussed (*Chem. and Ind.*, 1942, **61**, 273) the light absorption of geometrical isomerides and its implications in relation to the stereochemical structure of vitamin D. Some of the ideas emanating from this earlier work have since been extended in many laboratories and, in particular, mention might be made of a comparatively recent joint publication (*J.*, 1949, 1890) concerned with the development of the concept of steric inhibition of resonance in natural and synthetic derivatives of *cyclo*hexene.

In 1942, Koch joined the British Rubber Producers' Research Association and proceeded to apply spectroscopic methods of analysis and structural investigation to a variety of chemical problems. Naturally concerned at first with ultra-violet absorption, he later—after a period of secondment to Dr. G. B. B. M. Sutherland's laboratory at Cambridge—installed an infra-red spectrometer, and the resulting measurements and their interpretations became his primary interest from about 1947 onwards. The definitive value of spectroscopic data in his new sphere was soon apparent; his first joint publication from the Welwyn laboratories (*J.*, 1943, 472) established conclusively, and far more simply than could be done by chemical means, that chromans were the products of reaction between olefins and saligenin. Subsequently his work fell roughly into three groups: (i) the determination of the ultra-violet and infra-red spectra of olefins and related compounds, and their correlation in terms of electronic and stereochemical structural factors; (ii) the essentially analytical problems concerned with tracing double-bond displacements during olefinic reactions; and (iii) an extensive spectroscopic study of organic sulphur compounds. Notable work in the first group is that on glutaconic ester derivatives (*J.*, 1945, 216), on dicinnamyl and related compounds (*J.*, 1948, 1111, 1118, 1123), and a comprehensive investigation of double-bond structure in simple acyclic terpenes (*J.*, 1950, 915). The second group includes studies of the isomerisations accompanying autoxidation (*J.*, 1943, 541; 1945, 445) and bromination with *N*-bromosuccinimide (*J.*, 1950, 936, 3051). The third group comprises impressive contributions to our knowledge of the spectral and structural properties of a diverse collection of sulphides, sulphoxides, and sulphones (*J.*, 1949, 387, 394, 401, 408, 2442; *Trans. Faraday Soc.*, 1951, **47**, 7). Quite a different subject was discussed in his last paper, *viz.*, the infra-red spectra of tropolone and some of its derivatives and their constitutional requirements (*J.*, 1951, 512).

Koch was appointed to a senior lecturership at Manchester University from January 1951, but he did not commence his duties until March. He had been collaborating with workers in the Organic Chemistry Department there for some time previously in spectrographic studies resulting in the publication of a detailed paper on the infra-red spectra of a wide range of unsaturated steroids (*J.*, 1951, 2402). Although at Manchester University for only a few weeks, Koch had already made a most favourable impression, both scientifically and personally. His exceptional perspicacity over a broad field of scientific interest was a source of admiration to his co-workers and other colleagues and will long be remembered by them.

L. BATEMAN.
E. R. H. JONES.

ALEXANDER McKENZIE, M.A., D.Sc., LL.D., F.R.S.

1869—1951.

ALEXANDER MCKENZIE was born in Dundee on December 6th, 1869. His father, Peter Mitchell McKenzie was a good representative of the old type of Scottish " dominie," a man with varied interests. The boy's early education was received in his father's school first in Dundee, and then at Tealing. In 1882 he went to the High School of Dundee, having already made a start in Latin, Greek, French, and Mathematics. He joined the " classical " side of the school and, in 1885, was the Edinburgh Angus Club Medallist in Latin. When only 15 years old, he entered United College, St. Andrews, and laid a sure foundation for his liberal outlook of later life by reading for the pass M.A. degree. Gradually, particularly under the influence of Purdie, his scientific interest developed. He graduated B.Sc. in 1891 with Chemistry and Natural Philosophy as principal subjects. He was placed in the First Rank of Honours in nearly all his subjects. The thrill of research soon captivated him and, after working under Purdie for a while he gave up, at considerable personal sacrifice, his lectureship at United College to work with Marckwald in Berlin. He graduated Ph.D in 1901, and shortly afterwards graduated D.Sc. at St. Andrews. From February 1901 until October 1902 he held the Grocers' Company's Research Studentship in the Lister Institute, during which period he was engaged entirely in research in Harden's laboratory. From 1902 to 1905 he worked at Birmingham University under Professor P. F. Frankland, becoming Special Lecturer in Organic Chemistry. In 1905 he was appointed to the Headship of the Chemistry Department of Birkbeck College, London. He brought with him a well-considered plan of research and quickly gathered round him an enthusiastic band of co-workers at first drawn mainly from his younger colleagues and gradually augmented by senior students as the department developed. From 1914 to 1938 McKenzie was Professor of Chemistry in University College, Dundee. His researches expanded and developed throughout the field of stereochemistry and his reputation became world-wide. At home, the merit of his work was recognised by his election in 1916 as a Fellow of the Royal Society, and in Germany by the rare distinction of his admission as an Honorary Fellow of the Kaiserliche Deutsche Akademie der Naturforscher, Halle. On his retirement he received the LL.D. of St. Andrews. His interest in Chemistry continued after his retirement. He died peacefully, sitting in his armchair in the study, surrounded by the books he loved so much.

Another lifelong interest which McKenzie acquired at St. Andrews was golf. He became Secretary of the Golf Team. When in Berlin he won his way into the final for the championship of Germany and, in 1903, he was a semifinalist in the Amateur Scottish Golf Championship at Monifieth.

McKenzie was an exceedingly able organiser and administrator. His lectures were lucid and interesting and always brought very carefully up to date. His relationships with his research students were very happy and he had an instinctive knowledge of the amount of help and encouragement which each required. He took a lively and enduring interest in the personalities and progress of his pupils, an interest which he maintained throughout his years of retirement.

Professor McKenzie was married in 1906 to Miss Alice H. Sand and there is one son, Duncan Buchanan, who is with the Ministry of Supply.

McKenzie's researches were mainly concerned with stereochemical problems. His inaugural dissertation (Berlin, 1901) has become a classic, and if the suggested resolution of a racemic acid by partial esterification with an optically active alcohol is due to Marckwald, it may be justly claimed that the ultimate isolation of pure (—)-mandelic acid by this method could not have been achieved without the intimate knowledge of the chemistry of the acid which McKenzie acquired at St. Andrews. The counterpart of this method, the fractional hydrolysis of (—)-menthyl (±)-mandelate by alkali, proved a more difficult problem, involving processes of racemisation which were not understood until several years later. This work was subsequently extended to the partial resolution of (±)-*sec.*-octyl alcohol by (+)-tartaric acid, of (±)-mandelic acid by (—)-borneol, of (±)-α-naphthylglycollic acid and of (±)-*o*-nitromandelic acid by (—)-menthol; to the partial hydrolysis of (—)-bornyl (±)-lactate and other esters.

The value of McKenzie's work on the Walden inversion lies on the experimental side in showing how very general the action is and how it can be applied to different types. His work in the mandelic acid group was followed by the interconversion of the optically active atrolactinic acids which proved that the Walden inversion can occur with a compound which

has no hydrogen atom in direct attachment to the asymmetric carbon atom. Further important results include the proof of the difference in the action of phosphorus pentachloride and thionyl chloride in displacing hydroxyl, the observation that a Walden inversion can occur with an optically active ester, with a β-hydroxy-acid, and with an optically active alcohol. The " displacement racemisation " which occurs to a greater or less extent when a group attached to an asymmetric carbon atom is substituted by another group was examined in considerable detail and the semipinacolinic change was brought into the sphere of the Walden inversion. A hypothesis of the mechanism of the Walden inversion was based on the conception that addition precedes substitution and that an optically active structure containing a tervalent carbon atom can possess a transient existence without losing its optical activity. Several examples of such retention were observed.

McKenzie contributed greatly to the literature of catalytic and displacement racemisation. He brought much experimental evidence of the intimate relationship between keto-enolic desmotropy and racemisation by alkali, adduced examples of the racemisation of esters before their hydrolysis, and established the great susceptibility of acid amides. (Had he known at that time how readily the optically active mandelamides are racemised, it is doubtful if he would have attempted the preparation of the optically active benzoins.) He made many studies of the action of nitrous acid on amino-alcohols and of the dehydration of glycols.

A chance observation that 1-amino-2-hydroxy-1 : 2 : 2-triphenylethane is converted by nitrous acid, not into the corresponding glycol but into phenyldeoxybenzoin led McKenzie to examine the migration of hydrocarbon radicals in optically active compounds, a process which he named " semipinacolinic deamination." The most interesting application is that for the preparation of optically active ketones. A further important application lies in its use for contrasting the migrational aptitude of two different hydrocarbon radicals.

McKenzie's first asymmetric synthesis was that of (−)-atrolactinic acid, carried out in Birmingham in 1904. This was followed by many further examples in which he used mainly reduction, oxidation, and the Grignard reaction. He gave many examples of asymmetric induction. Incidental to his work were many resolutions of acids, amino-alcohols, bases, and esters, some of which proved very difficult experimentally.

McKenzie's quick appreciation of the value of the work of other chemists led him to be one of the first British scientists to use the Grignard reagents. The application of them in asymmetric syntheses has already been noted (see above). The preparation of (−)-benzoin from (−)-mandelamide opened a fruitful field and was followed by that of other acyloins on similar lines. The conversion of (−)-phenylchloroacetic acid into (+)-diphenylsuccinic acid is interesting by reason of the almost complete retention of optical activity. The reagent was also used for the preparation of the diethylamides of α-hydroxycarboxylic acids and of optically active and inactive glycols.

Among McKenzie's unclassified researches two may be mentioned. The isolation of pure, optically active amyl alcohol from fusel oil made a much-needed addition to the number of optically active alcohols available for stereochemical research. The examination of the resolution of ammonium racemate by moulds showed that, contrary to the then-prevalent idea, both active components are attacked simultaneously but at different rates.

H. Wren.

WALTER COLLINGWOOD WILLIAMS.

1863—1951.

Walter Collingwood Williams died on April 5th, 1951, in his 89th year; by his death the chemical profession has lost probably the last of the original pioneers who " blazed the trail " of the Public Analyst.

He was apprenticed to one of the London Guilds and, after serving his full term, studied at London University, obtaining his B.Sc. degree with honours in chemistry and physics. He then attended Mason College, Birmingham, and was elected Associate of the College in 1884.

On leaving Birmingham he became an assistant to Dr. J. Campbell Brown and shortly afterwards was appointed, jointly with Dr. Brown, Public Analyst for Lancashire, Liverpool, and several boroughs. On the death of his chief in 1910 he became whole-time Public Analyst for Lancashire, a post which he held until his retirement in 1926.

He was elected a Fellow of the Society in 1885 and became a Fellow of the Institute of Chemistry in 1893.

In the early days of his career the post of Public Analyst had only recently been established. Very little attention had been given to the composition of foods or to the detection of adulteration. Hassall had published his classical researches on microscopy applied to food stuffs but no corresponding work had appeared relating to analytical investigations. Consequently in the Lancashire County Laboratory, as in other laboratories, research was carried out to obtain information regarding the composition of foods and the detection of adulteration, and to establish limits and standards. Williams threw himself whole-heartedly into this work; his enthusiasm was such that it stimulated the deep and permanent interest in those who served under him.

A vast amount of information was accumulated as the years passed but little or none of it was published with the exception, it is believed, of a paper on Jamaica rum. Had his policy been to make known the results of his work he would have occupied a much more prominent position than he did in the profession.

Williams had a very reserved temperament, and a sense of humour, though very seldom did evidence of it emerge in working hours. In this connection it should, in fairness, be mentioned, that at the period usually regarded as the prime of life his eyesight began to fail and it seems quite likely that this misfortune would emphasise a disposition that had always been a retiring one.

The chief recreation of his active life was mountaineering and he scaled many peaks on the continent and in Britain. He was very much attracted to all mechanical contrivances; microscopy claimed much of his spare time and he attained a wide and thorough knowledge of the subject. He was a devoted member of the Liverpool Microscopical Society.

N. Heron.

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